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Proceedings of the Fifth Annual Chemical Defense Bioscience Review

US Army Medical
Research and Development Command

Appendix 3

Sponsored by the

US Army Medical Research
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29 - 31 May 1985

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Appendix 3

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11. PERFORMANCE.

8. Skin Injury and Protection

THE ISOLATED PERFUSED PORCINE SKIN FLAP: A NOVEL IN VITRO ANIMAL MODEL SYSTEM FOR DRUG AND XENOBIOTIC PERCUTANEOUS ABSORPTION STUDIES

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INTRODUCTION:

PERCUTANEOUS ABSORPTION AND CUTANEOUS TOXICOLOGY STUDIES HAVE TRADITIONALLY BEEN CONDUCTED IN HUMAN AND LABORATORY ANIMALS IN VIVO AND IN VITRO USING DIFFUSION CHAMBERS AND CELL CULTURE TECHNIQUES. STUDIES IN VITRO ARE CONDUCTED USING DEAD SKIN PREPARATIONS IN DIFFUSION CELLS WHERE CHEMICALS ARE APPLIED ONTO THE SURFACE OF THE EPIDERMIS AND ABSORPTION ASSESSED BY MONITORING THE APPEARANCE OF THE APPLIED CHEMICAL IN A RECEPTOR FLUID IN CONTACT WITH THE DERMAL SIDE OF THE PREPARATION. IN VITRO CUTANEOUS TOXICOLOGY STUDIES HAVE BEEN LARGELY LIMITED TO CELL CULTURE TECHNIQUES. IN VIVO ANIMAL STUDIES HAVE UTILIZED ALL SPECIES OF LABORATORY ANIMALS AND IN GENERAL MINIATURE AND WEANLING SWINE, AS WELL AS THE MONKEY, APPEAR TO CLOSELY MODEL PERCUTANEOUS ABSORPTION IN HUMANS FOR A WIDE VARIETY OF DRUGS AND XENOBIOTICS. HOWEVER, THERE ARE A NUMBER OF PROBLEMS IN CUTANEOUS TOXICOLOGY AND PHARMACOLOGY FOR WHICH SATISFACTORY ANIMAL MODELS HAVE NOT BEEN DEVELOPED.

STUDIES OF PERCUTANEOUS ABSORPTION UTILIZING IN VITRO DIFFUSION CELLS ARE NOT OPTIMAL IF THE DRUG IS HIGHLY LIPOPHILIC AND ARE NOT APPROPRIATE WHEN THE DRUG UNDERGOES CUTANEOUS BIOTRANSFORMATION SINCE A VIABLE EPIDERMAL CELL LAYER IS REQUIRED. IN CONTRAST, IN VIVO STUDIES OF CUTANEOUS BIOTRANSFORMATION ARE CONFOUNDED BY SYSTEMIC METABOLISM OF THE ABSORBED DRUG IN THE LIVER AND KIDNEY. IF THE COMPOUND IS A POTENT SYSTEMIC TOXIN, IN VIVO STUDIES ARE NOT

FEASIBLE. PRESENTLY UTILIZED IN *VIVO* TECHNIQUES DO NOT ALLOW FOR THE COLLECTION OF DRUG ABSORPTION AND CUTANEOUS DISPOSITION DATA WHICH CAN READILY BE UTILIZED IN PREDICTIVE PHARMACOKINETIC MODELS.

AN ISOLATED, PERFUSED VIABLE SKIN PREPARATION WOULD OVERCOME MANY OF THESE LIMITATIONS SINCE COMPOUND ABSORPTION, BIOTRANSFORMATION AND DISPOSITION TO SKIN COULD BE PRECISELY MONITORED WITHOUT INTERFERENCE FROM SYSTEMIC PROCESSES. EARLIER ATTEMPTS AT ISOLATED SKIN PERFUSION WERE DESCRIBED IN CANINE SKIN HOWEVER THE DOG IS NOT A SUITABLE SPECIES FOR PREDICTING PERCUTANEOUS DRUG AND XENOBIOTIC ABSORPTION IN MAN. IN ORDER TO OVERCOME THIS LIMITATION, WE DESCRIBE THE DEVELOPMENT OF AN ISOLATED PERFUSED PORCINE SKIN FLAP (IPPSF) WHICH WOULD BE AN OPTIMAL PREPARATION FOR EXPERIMENTALLY STUDYING MANY PROBLEMS FOR WHICH PRESENT TECHNIQUES ARE NOT OPTIMAL.

THESE INCLUDE: 1. MODELLING PERCUTANEOUS ABSORPTION OF DRUGS AND XENOBIOTICS WHICH HAVE A HIGH DEGREE OF SYSTEMIC TOXICITY, ARE HIGHLY LIPOPHILIC, OR UNDERGOE "FIRST-PASS" CUTANEOUS BIOTRANSFORMATION; 2. ASSESSING THE EFFECTS OF CHANGING BLOOD FLOW OR ALTERED EPIDERMAL METABOLISM ON PERCUTANEOUS ABSORPTION PROCESSES; 3. PROVIDE A MECHANISM BY WHICH THE SKIN'S CONTRIBUTION TO DRUG OR XENOBIOTIC DISPOSITION (ORGAN SPECIFIC CLEARANCE AND VOLUME OF DISTRIBUTION) CAN BE DETERMINED FOR INPUT INTO PHYSIOLOGIC PHARMACOKINETIC MODELS; 4. DEFINING ABSORPTION PROFILE OF DRUGS AND PRODRUGS ADMINISTERED IN A TRANSDERMAL DELIVERY SYSTEM; 5. IDENTIFY SKIN - SPECIFIC BIOCHEMICAL MARKERS OF CUTANEOUS TOXICITY; AND 6. DETERMINE THE CONCENTRATION OF DRUG

OR XENOBIOTIC IN PERFUSATE NECESSARY TO ILLICIT A SPECIFIC PHARMACOLOGIC OR TOXICOLOGIC EFFECT IN THE SKIN.

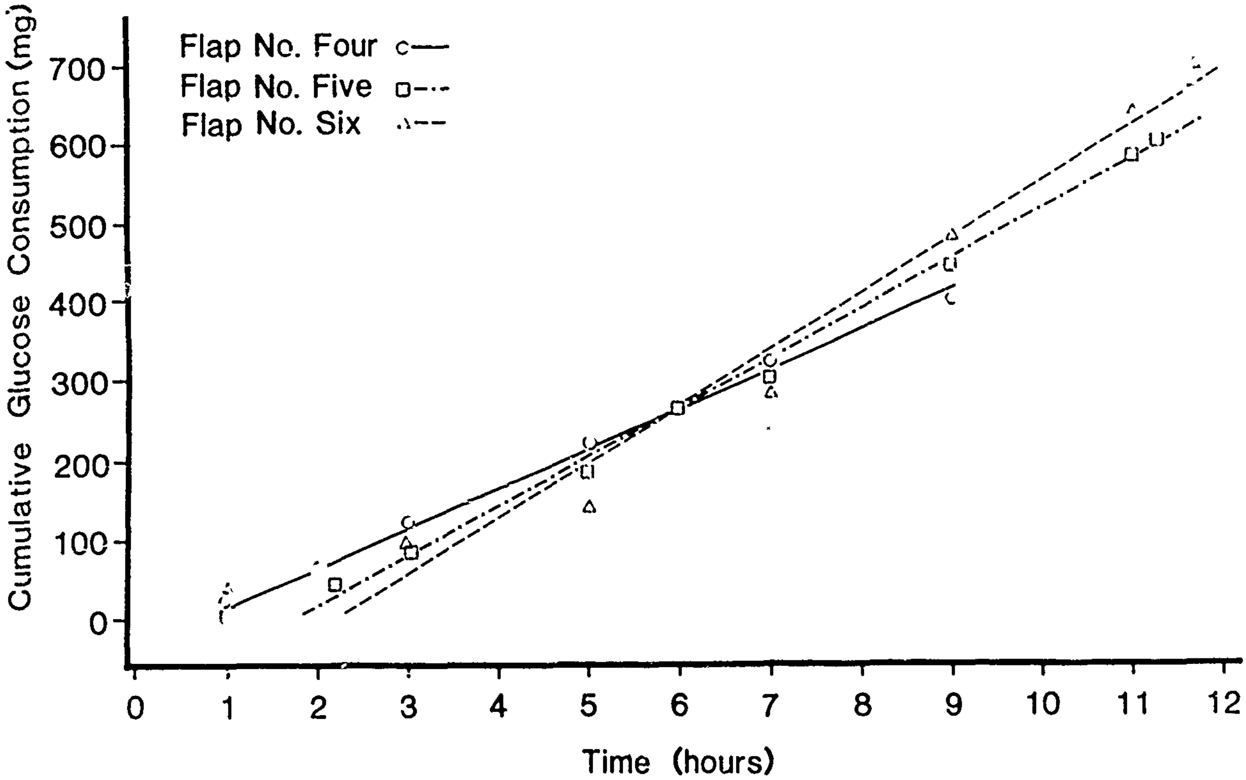

FIGURE 2: PLOT OF CUMULATIVE GLUCOSE CONSUMPTION (MG) VERSUS TIME (HRS.) FOR THREE IPPSF'S. THE SYMBOL USED IS FLAP NUMBER. LINES ARE THE LEAST SQUARES FIT.

MATERIALS AND METHODS:

SIX SINGLE-PEDICLE, AXIAL-PATTERN, TUBED SKIN FLAPS BASED ON THE CAUDAL SUPERFICIAL EPIGASTRIC ARTERY WERE LIFTED (STAGE ONE SURGICAL PROCEDURE) ON YORKSHIRE WEANLING PIGS IN THE MANNER DESCRIBED BY THE ACCOMPANYING PRESENTATION (BOWMAN ET AL. - SURGICAL TECHNIQUES). A TUBED FLAP WAS SELECTED IN ORDER TO HAVE THE MAXIMAL RATIO OF SURFACE AREA TO EXPOSED SUBCUTANEOUS TISSUE, AN OPTIMAL SITUATION IN PERCUTANEOUS DRUG ABSORPTION STUDIES. THE RAISED FLAP IS SUPPLIED PRIMARILY BY THE CAUDAL SUPERFICIAL EPISGASTRIC ARTERY WITH VENOUS DRAINAGE PROVIDED BY ITS ASSOCIATED PAIRED VENAE COMMITANTES. THIS TUBED FLAP WAS HARVESTED SIX TO 14 DAYS LATER (STAGE TWO SURGICAL PROCEDURE) BY CANNULATION OF THE ARTERY AND VEIN (WHEN ACCESSABLE) AND THEN TRANSFERRED TO THE ISOLATED PERFUSION APPARATUS DEPICTED IN FIGURE 1. THE PIG WAS RETURNED TO ITS PRIOR EXISTENCE AFTER THE DONOR SITE HEALED COMPLETELY. THE PERFUSION APPARATUS IS A CLOSED, RECIRCULATING SYSTEM OPTIMIZED FOR THE RELATIVELY LOW PERFUSATE FLOW RATES OF 0.5 TO 2.5 ML/MIN/FLAP OBSERVED. THIS APPARATUS IS ENCLOSED IN A HUMIFIED PLEXIGLASS CHAMBER MAINTAINED AT A TEMPERATURE OF 37'C. PERFUSATE IS GASSED WITH A 95% OXYGEN AND 5% CARBON DIOXIDE MIXTURE USING A SILASTIC-TUBE OXYGENATOR, APPROXIMATELY 400 MM/KG. TWO TO THREE HUNDRED ML OF MEDIA ARE CIRCULATED TO THE CANNULATED ARTERY OF THE SKIN FLAP WITH A VARIABLE RATE PERISTALTIC PUMP. MEDIA IS ALSO RECIRCULATED AT A HIGHER FLOW RATE BETWEEN ARTERIAL AND VENOUS RESERVOIRS. GLUCOSE MAY BE ADDED TO THIS RECIRCULATING SHUNT LINE TO MAINTAIN DEFINED GLUCOSE CONCENTRATIONS IN THE PERFUSATE. ARTERIAL PERFUSATE PRESSURE, TEMPERATURE, FLOW,

GLUCOSE, OSMOLARITY, LACTATE, LACTATE DEHYDROGENASE (LDH) AND VENOUS GLUCOSE AND PH WERE MONITORED TO ASSESS VIABILITY. PERFUSATE CONSISTS OF A KREBS-RINGER BICARBONATE BUFFER SOLUTION (PH = 7.4, 330MOSM) CONTAINING BOVINE SERUM ALBUMIN (COHN FRACTION V), GLUCOSE, SODIUM HEPARIN, GENTAMICIN, PENICILLIN G AND AMPHOTERICIN. SINCE SKIN IS NOT A STERILE ORGAN, ANTIBIOTICS MUST BE INCLUDED IN THE PERFUSATE TO PREVENT BACTERIAL OVERGROWTH. AFTER 11 HOURS OF VIABLE PERFUSION IN TWO FLAPS, SODIUM FLUORIDE, AN INHIBITOR OF GLYCOLYSIS, WAS ADDED TO THE PERFUSATE TO ASSESS THE PREPARATIONS SENSITIVITY TO METABOLIC INHIBITION. MICROANGIOGRAPHY WAS USED TO ASSESS THE IPPSF'S MICROCIRCULATION. AT THE TERMINATION OF AN EXPERIMENT, SKIN SAMPLES WERE COLLECTED FOR BOTH LIGHT AND TRANSMISSION ELECTRON MICROSCOPY. FOR LIGHT MICROSCOPY TISSUES WERE FIXED IN
TEN PERCENT NEUTRAL BUFFERED FORMALIN AND ROUTINELY PROCESSED AND STAINED WITH HEMATOXYLIN AND EOSIN. TISSUES FOR ELECTRO MICROSCOPY WERE FIXED IN 2.5% GLUTARAI EHYDE AND 2% PARAFORMALDEHYDE IN 0.2M CACODYLATE BUFFER (976 MOSM) AND EMBEDDED IN SPURR'S RESIN.

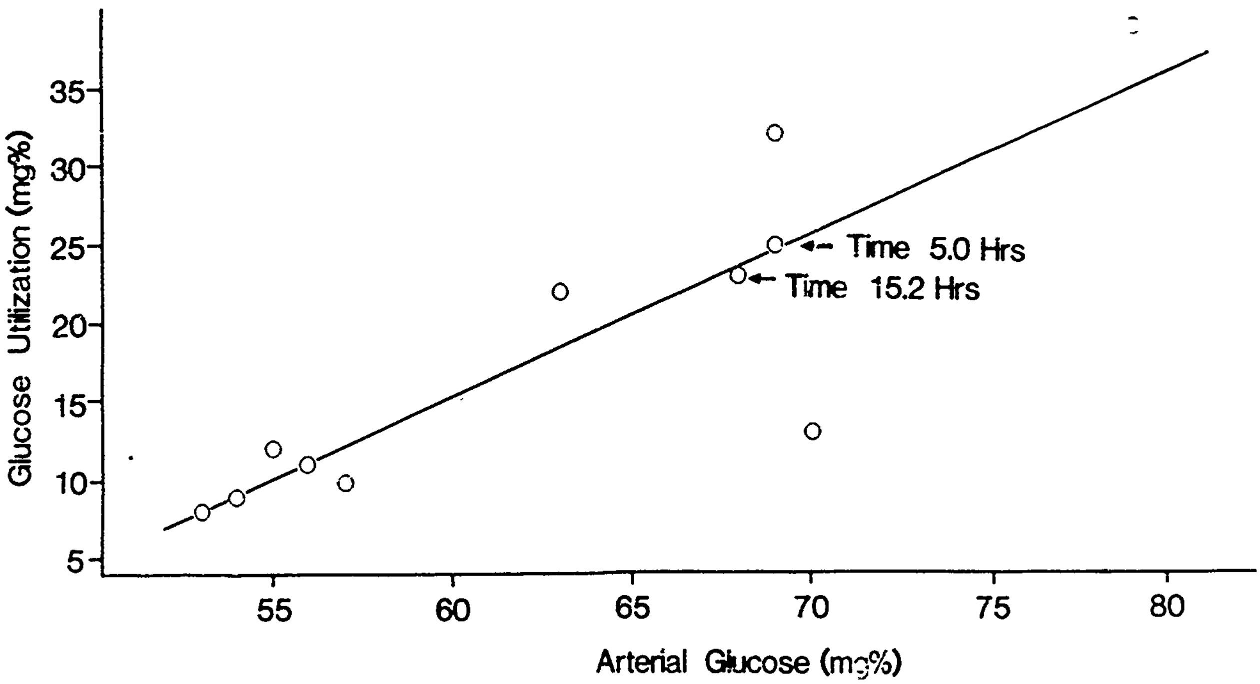

FIGURE 3: GLUCOSE UTILIZATION VS. ARTERIAL PERFUSATE CONCENTRATION IN FLAP 2.

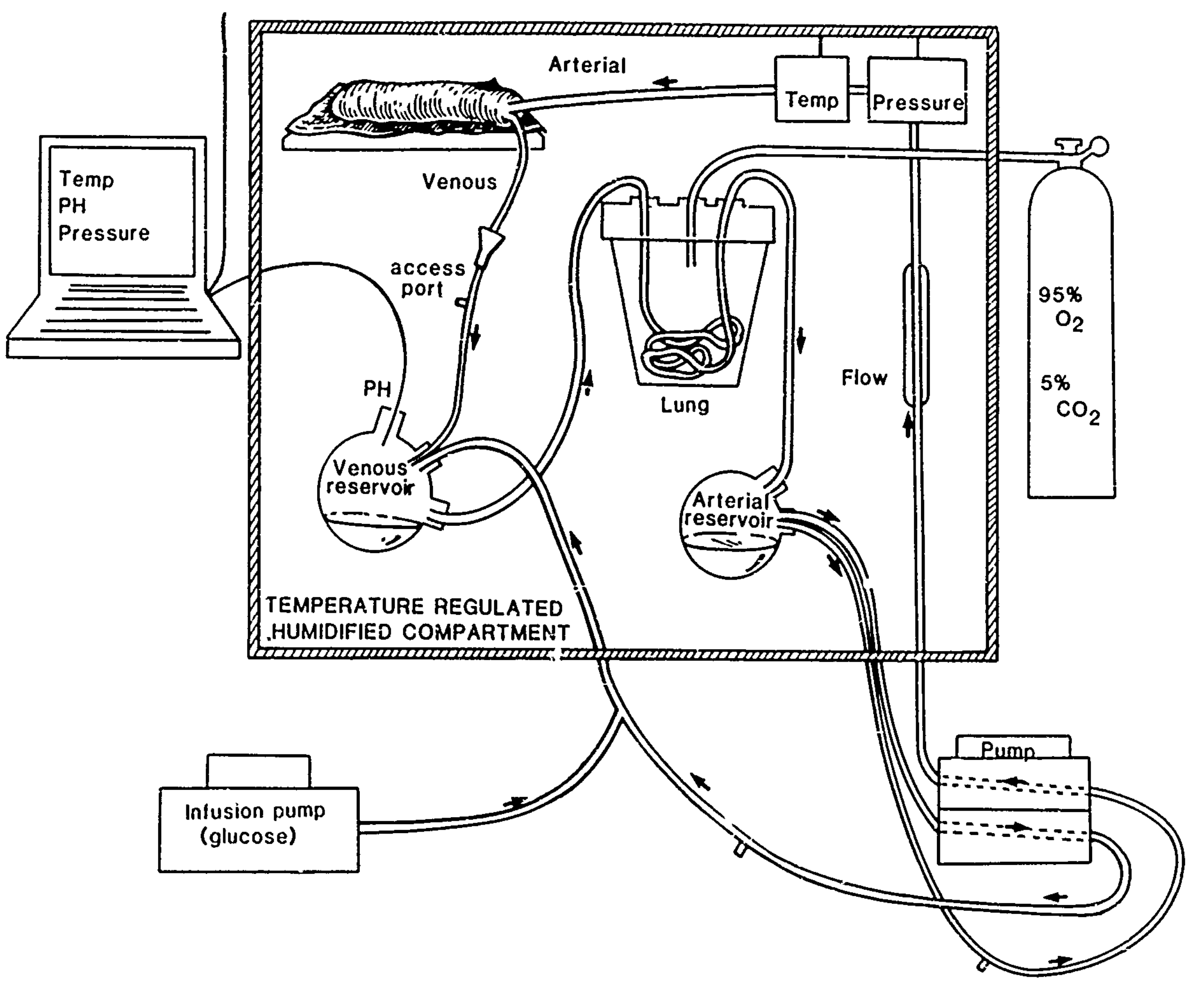

FIGURE 1: SCHEMATIC OF THE ISOLATED PERFUSED SKIN FLAP APPARATUS.

FIGURE 4: LIGHT MICROGRAPH (H&E) DEPICTING NORMAL VIABLE EPIDERMIS TAKEN FROM FLAP 2.

FIGURE 5: A. TRANSMISSION ELECTRON MICROGRAPH SHOWING VIABLE EPIDERMAL CELLS AND NUCLEOLAR PLEOMORPHISM (ARROW) IN THE STRATUM BASALE (SB) AND STRATUM SPINOSUM (SS) LAYERS. X5,400
B. TEM OF THE SAME FLAP SHOWING NORMAL STRATUM GRANULOSUM (SG).

TABLE I: GLUCOSE UTILIZATION IN IPPSF's (a)

FLAP	1	2	3	4	5	6
ARTERIAL GLUCOSE (mg%)	96 ± 23	63 ± 8	110 ± 7	155 ± 26	94 ± 8	86 ± 16
GLUCOSE EXTRACTION (%)	30 ± 13	28 ± 12	18 ± 6	9 ± 4	28 ± 14	17 ± 6
GLUCOSE UTILIZATION (mg% - ml/min)	23 ± 20	7 ± 6	22 ± 8	28 ± 8	32 ± 12	40 ± 22
CUMULATIVE GLUCOSE CONSUMPTION (mg)	617	160	662	445	656	695
PERFUSATE FLOW (ml/min)	1.2±1.4	0.4±0.1	1.2±0.3	1.7±0.2	1.5±.6	2.4±0.3
VIABLE PERIOD (hrs.) (b)	16	15	12	12	11(c)	11(c)

(a) Mean ± SD

(b) Cessation of viability marked by decreased glucose extraction, decreased perfusate flow and increased perfusate LDH activity.

(c) NaF administered after 11 hrs. Glucose extraction dropped to 2% and perfusate flow rate increased 3 to 7 fold.

FIGURE 6: ELECTRON MICROGRAPH OF PIG EPIDERMIS TAKEN FROM A TWELVE HOUR SAMPLE OF DEAD SKIN. NOTE DAMAGED MITOCHONDRIA (ARROWS) AND SINGLE VACUOLES (V). 8,800X

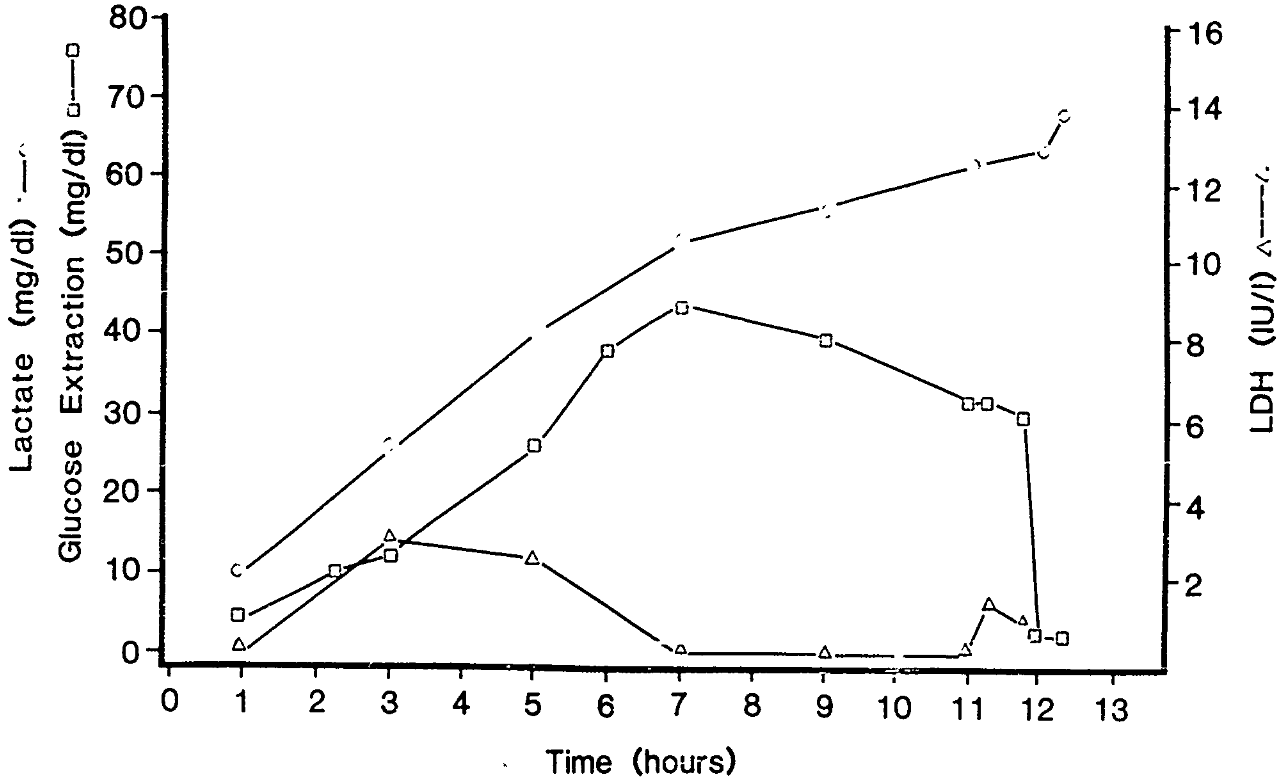

FIGURE 7: PLOT OF GLUCOSE EXTRACTION (MG%), LDH (IU/L), AND LACTATE (MG/DL) VERSUS TIME (HOURS) FOR IPPSF 5. AFTER NAF AT 11 HRS, GLUCOSE UTILIZATION DROPPED TO 1 MG% AT 12 HRS.

RESULTS

TABLE I PRESENTS THE DATA SUMMARIZING GLUCOSE UTILIZATION IN THE SIX IPPSF PREPARATIONS WHICH REMAINED VIABLE FOR PERIODS OF 11 TO 16 HOURS. VIABILITY WAS DEFINED AS A PERIOD IN WHICH THERE WAS SIGNIFICANT GLUCOSE UTILIZATION, LACTATE PRODUCTION, A STABLE PERFUSATE FLOW RATE, AN ABSENCE OF SIGNIFICANT LDH CONCENTRATIONS IN THE PERFUSATE, AND AN ABSENCE OF DEGENERATIVE CHANGES ON LIGHT MICROSCOPIC EXAMINATION. GLUCOSE UTILIZATION (MG%-ML/MIN) WAS CALCULATED AS THE PRODUCT OF GLUCOSE EXTRACTION (MG%) AND THE PERFUSATE FLOW RATE (ML/MIN) AT EACH OBSERVATION TIME. THE CUMULATIVE GLUCOSE CONSUMPTION WAS ESTIMATED FROM THE AREA UNDER THE CURVE OF THE GLUCOSE CLEARANCE VERSUS TIME CURVE USING THE TRAPEZOIDAL RULE. IN ALL PREPARATIONS, CUMULATIVE GLUCOSE CONSUMPTION WAS LINEAR (R^2 BETWEEN 0.94 - 0.99) SUGGESTING UNIFORM GLUCOSE UTILIZATION OVER TIME (FIGURE 2). GLUCOSE EXTRACTION WAS LINEARLY CORRELATED WITH ARTERIAL GLUCOSE CONCENTRATION IN 4 OF 6 FLAPS (FIGURE 3). IN THREE FLAPS FOR WHICH LACTATE CONCENTRATIONS WERE MONITORED, ARTERIAL LACTATE INCREASED OVER THE TIME OF THE STUDY AND WAS LINEARILY CORRELATED ($R^2 > 0.92$) WITH CUMMULATIVE GLUCOSE UTILIZATION. LDH LEVELS WERE 6.1 ± 3.3 IU/L FOR A VIABLE PREPARATION AND INCREASED DRAMATICALLY (>30 IU/L) WHEN VIABILITY CEASED AS EVIDENCED BY A DECREASED PERFUSATE FLOW, A FALL IN GLUCOSE UTILIZATION AND PLATEAUING OF THE CUMULATIVE GLUCOSE CONSUMPTION CURVES. FIGURE 4 IS A LIGHT MICROGRAPH AND FIGURE 5 IS A TEM PHOTOGRAPH TAKEN FROM AN IPPSF DEPICTING NORMAL VIABLE EPIDERMIS. LOSS OF VIABILITY WAS MARKED BY DEGENERATIVE

CHANGES, AS DEPICTED IN THE TRANSMISSION ELECTRON MICROGRAPH OF DEAD SKIN COLLECTED FROM A SEPARATE VIABILITY STUDY (FIGURE 6). CELLULAR DEGENERATION MARKED BY SHRINKAGE OF THE NUCLEAR MEMBRANE, DAMAGED ORGANELLES AND SINGULAR, SMALL VACUOLES NOT ALWAYS DELINEATED BY A MEMBRANE CAN BE SEEN. WHEN SODIUM FLUORIDE (10 MG/ML) WAS ADMINISTERED AFTER 11 HRS OF VIABLE PERFUSION IN TWO FLAPS, GLUCOSE UTILIZATION CEASED WITHIN 40 MINUTES HOWEVER PERFUSATE FLOW RATE INCREASED (FIGURE 7). TRANSMISSION ELECTRON MICROSCOPY (FIGURE 8) ON THESE FLAPS REVEALED NORMAL EPIDERMIS EXCEPT FOR THE PRESENCE OF LARGE MULTIPLE VACUOLES, OFTEN MEMBRANE BOUND, IN THE STRATUM BASALE AND SPINOSUM LAYERS OF THE EPIDERMIS. FINALLY, MICROANGIOGRAPHY DEMONSTRATED THAT THE STRUCTURE OF THE DERMAL VASCULATURE WAS INTACT (FIGURE 9).

MICROGRAPH UNAVAILABLE

AT THE TIME OF PRINTING

FIGURE 8: SECTION OF SKIN FLAP WHICH RECEIVED SODIUM FLOURIDE AFTER 11 HOURS OF PERFUSION. LARGE MULTIPLE VACUOLES WERE NOTED (ARROWS). 4,800X

DISCUSSION

THESE EXPERIMENTS DEMONSTRATE THE FEASIBILITY OF MAINTAINING A VIABLE IPPSF FOR PERIODS OF 12 TO 16 HRS. AS JUDGED BY BIOCHEMICAL AND MORPHOLOGIC CRITERIA. THE ULTRASTRUCTURAL APPEARANCE OF THIS PERFUSED SKIN IS ESSENTIALLY NORMAL. CONSISTENT WITH OTHER STUDIES OF CUTANEOUS BIOCHEMISTRY, GLUCOSE UTILIZATION WAS DIRECTLY PROPORTIONAL TO THE AVAILABLE GLUCOSE CONCENTRATIONS AND LACTATE WAS A MEASURABLE END PRODUCT OF EPIDERMAL GLYCOLYSIS. SINCE SODIUM FLUORIDE, AN INHIBITOR OF GLYCOLYSIS AT THE LEVEL OF PHOSPHOENOLPYRUVATE FORMATION, STOPPED GLUCOSE UTILIZATION, THE INTEGRITY OF THE GLYCOLYTIC ENZYMATIC PATHWAY IS PROBABLE IN THIS PREPARATION. ADDITIONALLY, ACUTE TOXICITY CAUSED BY SODIUM FLUORIDE COULD BE EASILY DIFFERENTIATED FROM LOSS OF VIABILITY. BOTH ARE MARKED BY A DECREASED GLUCOSE UTILIZATION, HOWEVER TOXICITY RESULTED IN A DRAMATIC INCREASE IN FLOW RATE WHILE FLAP DEATH WAS MARKED BY A SLOWY DECREASING RATE. MORPHOLOGICAL APPEARANCE OF THESE TWO CONDITIONS WERE ALSO DISTINCT. THE AVERAGE GLUCOSE UTILIZATION DURING A VIABLE PERIOD WAS 28 ± 6 MG% - ML/MIN. IN FLAP 2, GLUCOSE UTILIZATION WAS LOWER AS WAS PERFUSATE FLOW RATE. HOWEVER, ARTERIAL GLUCOSE CONCENTRATIONS WERE ALSO LOW IN THIS PREPARATION. WHEN SUBSEQUENT FLAPS WERE INFUSED WITH GLUCOSE TO MAINTAIN A HIGHER AVERAGE GLUCOSE CONCENTRATION, UTILIZATION AND PERFUSATE FLOW WERE HIGHER. A "WORKING" DEFINITION OF A VIABLE SKIN FLAP PREPARATION WOULD BE ONE WITH A PERFUSATE FLOW RATE GREATER THAN 1.0 ML/MIN, A RATE OF GLUCOSE UTILIZATION AT LEAST 20 MG % - ML/MIN, AND LDH ACTIVITY, INDICATIVE OF LOSS OF CELLULAR MEMBRANE INTEGRITY, OF LESS THAN

APPROXIMATELY 30 IU/L. IT SHOULD BE NOTED THAT FOR THE INITIAL TWO HOURS AFTER HARVEST, GLUCOSE UTILIZATION IS SUPPRESSED, AS THE FLAP APPARENTLY RECOVERS FROM THE SURGICAL PROCEDURE AND "ADAPTS" TO THE PERFUSION APPARATUS. FUTURE STUDIES ARE BEING DESIGNED TO DETERMINE THE OPTIMAL PERFUSION MEDIA (AMINO ACIDS, KETOACIDS AS ENERGY SOURCES, ADDITION OF LIPOPROTEINS FOR DRUG ABSORPTION STUDIES).

AS OF THIS DATE, THE IPPSF PREPARATION APPEARS TO BE A PROMISING ANIMAL MODEL FOR STUDYING THE PROBLEMS OUTLINED ABOVE THE TUBED CONFIGURATION OPTIMIZES THE SURFACE AREA AVAILABLE FOR DRUG ABSORPTION, MINIMIZES EXPOSED SUBCUTANEOUS TISSUE WHICH COULD BE A SERIOUS SOURCE OF FLUID LOSS DURING PERFUSION, AND RESULTS IN AN INTACT, CLEAN, NONLEAKING PREPARATION IDEALLY SUITED FOR ISOLATED PERFUSION. THE ADVANTAGES OVER OTHER IN VITRO SYSTEMS ARE THE ANATOMICAL INTEGRITY OF THE PREPARATION IN RELATIONSHIP TO DERMAL MICROVASCULATURE AND CELLULAR CONSTITUENTS, THE PRESENCE OF A VIABLE EPIDERMAL CELL LAYER, AND THE ABILITY TO ASSESS ARTERIAL TO VENOUS DRUG AND XENOBIOTIC CONCENTRATIONS AS A FUNCTION OF PERFUSATE FLOW THROUGH THE SKIN. IN COMPARISON TO IN VIVO OR IN SITU SYSTEMS, PERFUSATE COMPOSITION CAN BE PRECISELY REGULATED, COMPOUNDS WITH A HIGH DEGREE OF SYSTEM TOXICITY CAN BE STUDIED AND EXPERIMENTS ARE NOT CONFOUNDED BY DISPOSITION OR BIOTRANSFORMATION OF THE COMPOUND IN OTHER ORGANS OR TISSUES OF THE BODY. ALSO NOTE THAT EXPERIMENTS MAY BE CONDUCTED WITH THIS PORCINE SKIN FLAP IN SITU, ALLOWING ARTERIAL AND VENOUS CONCENTRATIONS OF COMPOUNDS TO BE MONITORED WHILE ON THE ANIMAL. HOWEVER, THE IPPSF HAS AN ADVANTAGE IN THAT IT IS A HUMANE

PREPARATION SINCE ALL EXPERIMENTS ARE CONDUCTED AFTER FLAP HARVEST, ALLOWING THE PIG TO BE RETURNED TO ITS FORMER EXISTENCE.

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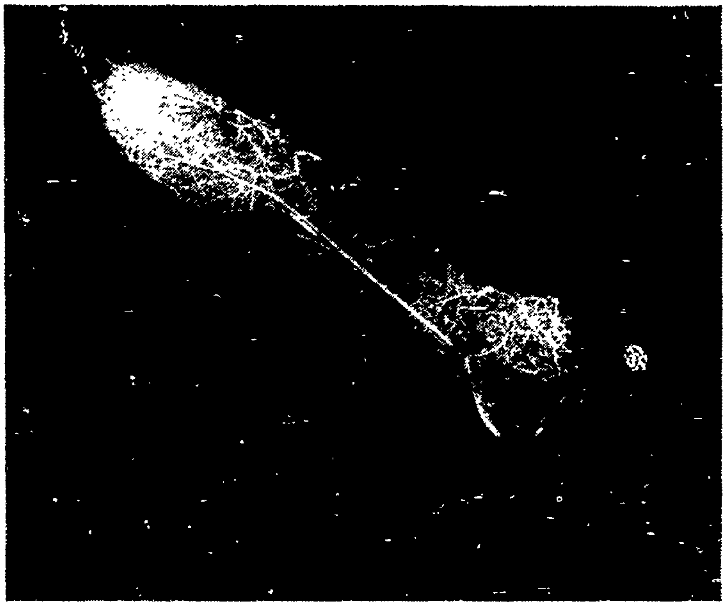

FIGURE 9: MICROANGIOGRAPH OF IPPSF 4 AFTER 12 HRS. FILLING OF CAUDAL SUPERFICIAL EPIGASTRIC ARTERY (LARGE ARROW) AND ITS DISTRIBUTION TO SUBCUTANEOUS AND DERMAL TISSUES IS OBVIOUS. THE CANNULATED VENAE COMMITANTES IS FILLED LESS OBVIOUSLY. NOTE THE RADIOPAQUE ARTERIAL CATHETER AND SKIN BIOPSY SITE.

DEVELOPMENT OF SURGICAL TECHNIQUES FOR PREPARATION OF IN VITRO ISOLATED PERFUSED PORCINE SKIN FLAPS FOR STUDY OF PERCUTANEOUS ABSORPTION OF XENOBIOTICS

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INTRODUCTION

THE DEVELOPMENT OF IN VITRO ISOLATED PERFUSED PORCINE SKIN FLAPS (IPPSF) FOR STUDY OF PERCUTANEOUS ABSORPTION OF XENOBIOTICS IS A MULTIPHASED, MULTIDISCIPLINARY PROJECT INVOLVING SPECIALISTS IN SURGERY, RADIOLOGY, ULTRASTRUCTURAL ANATOMY, TOXICOLOGY, AND PHARMACOLOGY. THE SURGICAL PHASE OF THIS PROJECT IS PRIMARILY CONCERNED WITH THE DEVELOPMENT AND STANDARDIZATION OF NECESSARY OPERATIVE PROCEDURES FOR THE ROUTINE HARVEST OF SKIN FLAPS FOR SUBSEQUENT IN VITRO PERFUSION.

BECAUSE OF THEIR DISCREET ARTERIAL AND VENOUS SUPPLY, INTERNAL ORGANS HAVE BEEN PERFUSED EASILY. ONE MUST ASSUME THAT THE LESS WELL-DEFINED BLOOD SUPPLY TO THE SKIN ACCOUNTS, IN PART, FOR THE CURRENT LACK OF AN ACCEPTABLE IN VITRO PERFUSED SKIN PREPARATION.

THE ARTERIAL BLOOD SUPPLY TO THE DERMAL-SUBDERMAL PLEXI OF SKIN WILL TRAVERSE 3 TYPES OF VASCULATURE: 1) THE SEGMENTAL; 2) THE PERFORATORS; AND 3) THE CUTANEOUS. IN GENERAL, THE VENOUS RETURN PARALLELS THE ARTERIAL SUPPLY. SEGMENTAL VESSELS ARISE FROM THE AORTA, LAY DEEP TO THE MUSCLE MASS, AND USUALLY FOLLOW THE COURSE OF A PERIPHERAL NERVE. PERFORATING VASCULATURE FUNCTIONS BY SUPPLYING BLOOD TO THE MUSCLES THROUGH WHICH THEY PASS AND SERVE AS CONDUITS FROM THE SEGMENTED VASCULATURE TO THE CUTANEOUS CIRCULATION.

THE CUTANEOUS ARTERIAL SUPPLY IS SUBDIVIDED INTO 2 TYPES: 1) THE MUSCULOCUTANEOUS ARTERIES; AND 2) THE

DIRECT CUTANEOUS ARTERIES. IN MAN AND PIG, THE MAIN BLOOD SUPPLY TO THE SKIN IS VIA MANY MUSCULOCUTANEOUS ARTERIES WHICH PENETRATE DIRECTLY FROM MUSCLE THROUGH THE SUBCUTANEOUS TISSUES AND INTO THE OVERLYING SKIN. MUSCULOCUTANEOUS ARTERIES SUPPLY RELATIVELY SMALL AREAS OF SKIN. THE MUSCULOCUTANEOUS ARTERIAL SYSTEM IS SUPPLEMENTED BY LIMITED NUMBERS OF ANATOMICALLY VARIABLE DIRECT CUTANEOUS ARTERIES WHICH COURSE PARALLEL, INSTEAD OF PERPENDICULAR, TO THE SKIN AT A LEVEL ABOVE THE MUSCLE AND FASCIA. DIRECT CUTANEOUS ARTERIES ARE GENERALLY ACCOMPANIED BY PAIRED VENAE COMMITANTES; THE DIRECT CUTANEOUS VEINS COURSE SUBDERMALLY. DIRECT CUTANEOUS ARTERIES SUPPLY MUCH GREATER AREAS OF SKIN.

A SKIN FLAP CONSISTS OF SKIN AND SUBCUTANEOUS TISSUE THAT IS MOVED FROM ONE PART OF THE BODY TO ANOTHER WITH A VASCULAR PEDICLE OR ATTACHMENT TO THE BODY BEING MAINTAINED FOR NOURISHMENT. SKIN FLAPS ARE CLASSIFIED BASED UPON THEIR BLOOD SUPPLY OR BY THE LOCATION TO WHICH THEY ARE MOVED (I.E., LOCAL OR DISTANT). PEDICLES OF RANDOM PATTERN FLAPS ARE USUALLY SUPPLIED BY MUSCULOCUTANEOUS ARTERIES, WHICH IN TURN PERFUSE THE DERMAL-SUBDERMAL PLEXI OF THE SKIN FLAP. THE SURVIVING LENGTH OF RANDOM PATTERN FLAPS IS RELATED TO THE ARTERIAL PERFUSION PRESSURE AND VENOUS DRAINAGE; HOWEVER, THESE SKIN FLAPS CAN BE MADE LARGER (50 TO 100 PERCENT), IF THEY ARE RAISED IN STAGES OR "DELAYED." WHEN A SKIN FLAP IS DESIGNED TO INCLUDE A DIRECT CUTANEOUS ARTERY WITHIN ITS LONGITUDINAL AXIS, THEY ARE CALLED AXIAL PATTERN FLAPS. AXIAL PATTERN FLAPS ARE FURTHER SUBCLASSIFIED AS: 1) PENINSULAR FLAPS, WHICH HAVE DIRECT CUTANEOUS

VASCULATURE WITH AN INTACT SKIN/SUBCUTANEOUS TISSUE BRIDGE; 2) ISLAND FLAPS, WHICH DO NOT HAVE A SKIN/SUBCUTANEOUS TISSUE BRIDGE BUT ARE ATTACHED TO A VASCULAR SUPPLY; AND 3) FREE FLAPS WHICH ARE TRANSPLANTED TO DISTANT SITES AND CONNECTED TO RECIPIENT VASCULATURE BY MICROVASCULAR ANASTOMOSIS

THE SURVIVING LENGTH OF AN AXIAL PATTERN FLAP IS DETERMINED BY THE EXTENT OF THE DIRECT CUTANEOUS ARTERY IN THE FLAP PLUS THAT DISTAL SKIN WHICH IS PERFUSED SUCCESSFULLY THROUGH ITS DERMAL-SUBDERMAL PLEXIS. ISLAND FLAPS SURVIVE TO AT LEAST THE SAME LENGTH AS PENINSULAR FLAPS WHEN BOTH ARE MADE UNDER SIMILAR CONDITIONS. AXIAL PATTERN FLAPS OFTEN SURVIVE TO APPROXIMATELY 50 PERCENT GREATER LENGTHS THAN "UNDELAYED" RANDOM PATTERN FLAPS; AND, AXIAL PATTERN FLAPS CAN BE MADE LONGER BY DELAY PROCEDURES.

AMONG THE SEVERAL TYPES OF DISTANT SKIN FLAPS IS THE TUBED FLAP. A TUBED FLAP IS A BI-EDICLED (I.E., ATTACHED AT BOTH ENDS) FLAP, WHICH HAS BEEN RAISED IN AN AREA OF ABUNDANT SKIN AND ITS PARALLEL EDGES SEWN TOGETHER TO FORM A TUBE RESEMBLING A SUITCASE HANDLE. THE SKIN BENEATH THE TUBED FLAP (I.E., DONOR SITE) IS EITHER UNDERMINED AND CLOSED BY PRIMARY SUTURE OR GRAFTED. THEREAFTER, THE TUBED FLAP CAN BE DETACHED ONE END AT A TIME AND "TUMBLED," "WALTZED," OR "CATERPILLARED" TO THE DISTANT, RECIPIENT SITE.

AXIAL PATTERN FLAPS HAVE BEEN RAISED SUCCE-SFULLY IN PIGS; HOWEVER, THE DIRECT CUTANEOUS ARTERY UPON WHICH THE SKIN FLAP IS BASED (I.E., THE LOCATION OF THE SKIN FLAP ON THE PIG'S BODY) WILL DICTATE ITS SURVIVING LENGTH. EXPERIMENTAL TUBED FLAPS HAVE BEEN RAISED ON PIGS FOR SKIN FLAP SURVIVAL

STUDIES; HOWEVER, WE WERE UNABLE TO LOCATE ANY REPORT DESCRIBING OR UTILIZING AXIAL PATTERN TUBED FLAPS IN THE PIG. SINGLE PEDICLE, AXIAL PATTERN TUBED FLAPS, BASED UPON THE INFERIOR (CAUDAL) SUPERFICIAL EPIGASTRIC ARTERY ARE USED IN RECONSTRUCTIVE SURGERY IN MAN.

OBJECTIVES

THE DEVELOPMENT OF SURGICAL TECHNIQUES FOR PREPARATION OF IPPSF REQUIRES THE CHARACTERIZATION OF A SINGLE-PEDICLE, AXIAL PATTERN TUBED SKIN FLAP WHICH CAN: 1) BE RAISED IN ONE OPERATION; 2) SURVIVE TO ITS ENTIRE LENGTH; 3) BE VALIDATED REGARDING ITS PHYSIOLOGICAL AND PHARMACOLOGICAL PROPERTIES DURING HEALING; AND, 4) BE HARVESTED SUBSEQUENTLY FOR TRANSFER TO THE IN VITRO PERFUSION APPARATUS. THE TUBED CONFIGURATION OF AN AXIAL PATTERN FLAP WAS SELECTED TO PROVIDE THE GREATEST RATIO OF SURFACE AREA TO EXPOSED SUBCUTANEOUS TISSUE DURING PERCUTANEOUS ABSORPTION STUDIES.

EXPERIMENTS IN PROGRESS

REFINEMENT IN ALL PHASES OF THE SURGICAL PROCEDURES ARE REQUIRED, ESPECIALLY DETERMINATION OF THE TIME(S) FOLLOWING STAGE 1 PROCEDURE WHEN THAT TUBED FLAP CAN BE HARVESTED. CURRENTLY, WE HAVE FOCUSSED OUR STUDIES ON CORRELATIVE EVALUATION OF MICROANGIOGRAPHY AND LIGHT AND ELECTRON MICROSCOPY IN TUBED FLAPS AT TIME 0, DAY 2, AND DAYS 4-6. THIS IS BEING DONE FOR 2 REASONS: 1) PRELIMINARY DATA ON SERIAL SKIN BIOPSIES OBTAINED DURING THE EARLY HEALING PHASES OF THE STAGE 1 PROCEDURE HAVE SHOWN HYPERPLASTIC CHANGES (I.E., NUCLEOLAR PLEOMORPHISM, INCREASED TONOFILAMENTS AND THICKENED EPIDERMIS); THEREFORE, IT MAY BE

NECESSARY TO STANDARDIZE THE TIMING OF OPERATIVE PROCEDURES BASED UPON AVAILABILITY OF MORE NORMAL SKIN, I.E., EITHER LESS THAN 7 DAYS BETWEEN STAGED PROCEDURES OR MUCH LATER; AND, 2) IF THE PROTOCOL CAN BE STANDARDIZED WITH THE LEAST AMOUNT OF TIME FROM TUBED FLAP PREPARATION TO HARVEST, THEN THE TOTAL COST OF THE EXPERIMENTAL MODEL IS MUCH LESS.

BASED UPON OUR EVALUATION OF CAUDOLATERAL EPIGASTRIC PENINSULAR AND ISLAND AXIAL PATTERN FLAPS, IN BOTH "FLAT", (I.E., RAISED AND SUTURED BACK TO ITS DONOR SITE), AND TUBED CONFIGURATION, WE HAVE FOUND THAT THERE IS ESSENTIALLY NO DIFFERENCE IN THEIR SURVIVING LENGTHS AT 7 DAYS AFTER SURGERY. THEREFORE, WE HAVE SELECTED RECENTLY THE ISLAND, AS OPPOSED TO PENINSULAR, CONFIGURATION OF AN AXIAL PATTERN TUBED FLAP AS BEING ABLE TO PROVIDE THE MOST OPTIMAL VASCULAR PATTERN FOR SUBSEQUENT IN VITRO ISOLATED PERFUSION. THIS APPEARS TO HAVE THE ADVANTAGE, AT LEAST DURING THE EARLY PHASES OF WOUND HEALING, OF ELIMINATING ACCESSORY VASCULATURE ACROSS THE SUTURED, BUT INCOMPLETELY REVASCULARIZED SKIN BRIDGE, THEREBY PROMOTING HYPERTROPHY OF THE VENAE COMMITANTES (I.E., SOLITARY VENOUS OUTFLOW) AND LESSENING SPURIOUS LEAKAGE OF PERFUSATE FROM THE WOUND MARGINS DURING SUBSEQUENT IN VITRO ISOLATED PERFUSION. OTHER REFINEMENTS NEEDED IN THE SURGICAL PROCEDURES INCLUDE IMPROVED METHODS OF VENOUS CANNULATION DURING THE STAGE 2 PROCEDURE.

INITIAL EXPERIMENTS

INITIAL EXPERIMENTS HAVE ESTABLISHED THE NORMAL VASCULATURE TO POTENTIAL SKIN FLAP DONOR SITES IN EMBALMED, LATEX-INJECTED PIGS (N=2, FIGURE 1); AND, IN LIVE PIGS USING SELECTIVE ANGIOGRAPHY (N=2, FIGURE 2). COMPARED TO PREVIOUSLY REPORTED SITES, OUR PROPOSED LOCATION (FIGURES 2 AND 3) HAS CONSISTENT DIRECT CUTANEOUS VASCULATURE, VIA THE CAUDAL SUPERFICIAL EPIGASTRIC ARTERY. FURTHERMORE, THE SKIN OF THIS REGION IS EASILY TUBED, ITS DONOR SITE CAN BE CLOSED BY PRIMARY SUTURE, AND THERE IS NO ASSOCIATED CUTANEOUS TRUNCI MUSCLE.

TO DATE, OUR STUDIES (N=26) HAVE INDICATED THAT A SINGLE-PEDICLE, AXIAL PATTERN TUBED FLAP CAN BE RAISED (STAGE 1 PROCEDURE) AND SURVIVE TO ITS ENTIRE LENGTH FOR AS LONG AS 29 DAYS. FURTHERMORE, OUR STUDIES HAVE INDICATED THAT IN THE SECOND STEP (STAGE 2 PROCEDURE), THE TUBED FLAP CAN BE RESECTED AND ITS AXIAL VASCULATURE CANNULATED (N=33); AND EITHER PREPARED FOR MICROANGIOGRAPHY OR TRANSFERRED TO THE IN VITRO ISOLATED PERFUSION APPARATUS (N=6, SEE COMPANION ABSTRACT, RIVIERE JE ET AL).

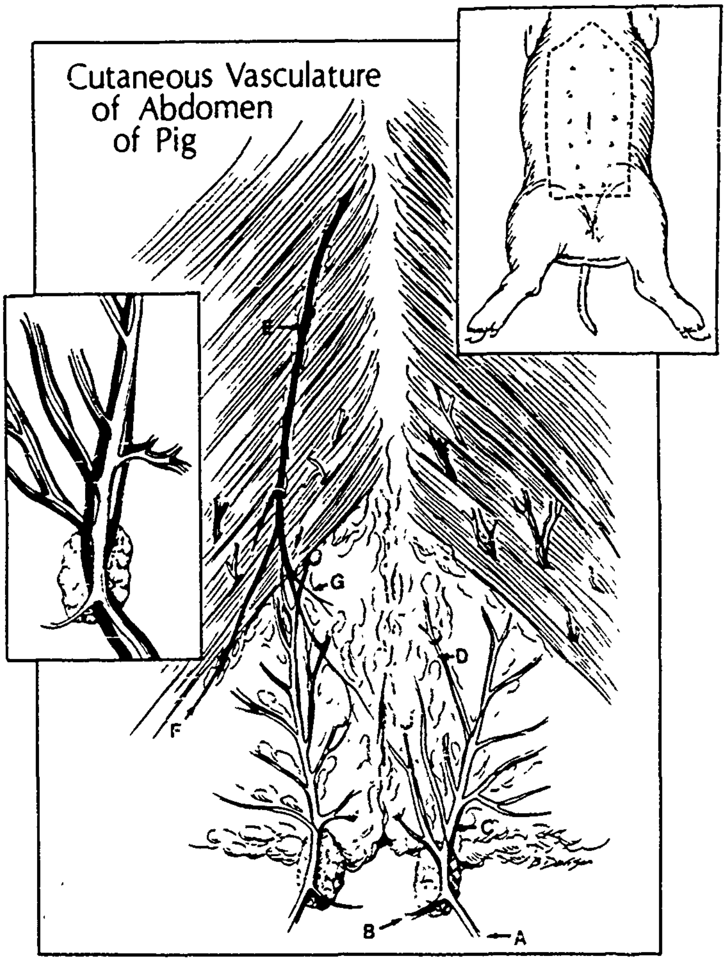

FIGURE 1: BOTH LEFT AND RIGHT CAUDAL SUPERFICIAL EPIGASTRIC (CSE) ARTERIES FOLLOW SIMILAR DISTRIBUTION PATTERNS: 1) ORIGIN (A); 2) CAUDOMEDIAL BRANCHES (B); AND, 3) CRANIAL EXTENSION WHICH DIVIDES INTO A LATERAL PORTION (C) SUPPLYING THE SKIN LATERAL TO THE LINE OF MAMMAE, AND CRANIOMEDIAL BRANCHES (D) SUPPLYING EACH OF THE CAUDAL MAMMAE AND MEDIAN SKIN. TWO VENOUS SYSTEMS WERE OBSERVED: 1) PAIRED VENAE COMMITANTES IN ASSOCIATION WITH THE CSE ARTERY (SEE DETAIL IN INSET); AND 2) A CRANIAL SUPERFICIAL EPIGASTRIC VENOUS SYSTEM (E) WITH AN ANASTOMOTIC BRANCH TO THE DEEP CIRCUMFLEX ILIAC VEIN (F) AND AN ILL-DEFINED CAUDAL EXTENT (G) THAT TERMINATES IN THE REGION OF THE CAUDAL MAMMAE. COMMUNICATIONS BETWEEN THESE VENOUS SYSTEMS HAVE BEEN OBSERVED.


B
C
A

FIGURE 2: SELECTIVE ANGIOGRAPHY OF THE RIGHT CAUDAL SUPERFICIAL EPIGASTRIC ARTERY IN PIG 4. STAINLESS STEEL SKIN STAPLES HAVE BEEN AFFIXED TO THE SKIN DENOTING LOCATION OF OUR PROPOSED CAUDOLATERAL EPIGASTRIC FLAP (CROSSED STAPLES) AND THE MIDLINE (SOLITARY STAPLES).

A - 1 SECOND AFTER INFUSION OF CONTRAST MEDIA. NOTE PRESENCE OF AXIAL ARTERY (ARROWS) APPROXIMATELY 1 CM LATERAL TO LINE OF MAMMAE FOR ENTIRE EXTENT OF FLAP. THERE ARE AT LEAST 2 CRANIOMEDIAL MEDIAL BRANCHES OF CSE ARTERY SUPPLYING THE CAUDAL MAMMAE AND THERE IS OBVIOUS ARTERIAL DISTRIBUTION TO LATERAL ASPECTS OF PROPOSED FLAP.

B - 5 SECONDS AFTER INFUSION OF CONTRAST MEDIA. NOTE FILLING OF THE CRANIAL SUPERFICIAL EPIGASTRIC VENOUS SYSTEM (A), THE ANASTOMOTIC BRANCH TO THE DEEP CIRCUMFLEX ILIAC VENOUS SYSTEM (B), AND THE CAUDAL EXTENSIONS WHICH DRAIN THE CAUDOLATERAL EPIGASTRIC AND MEDIAL THIGH REGIONS (C).

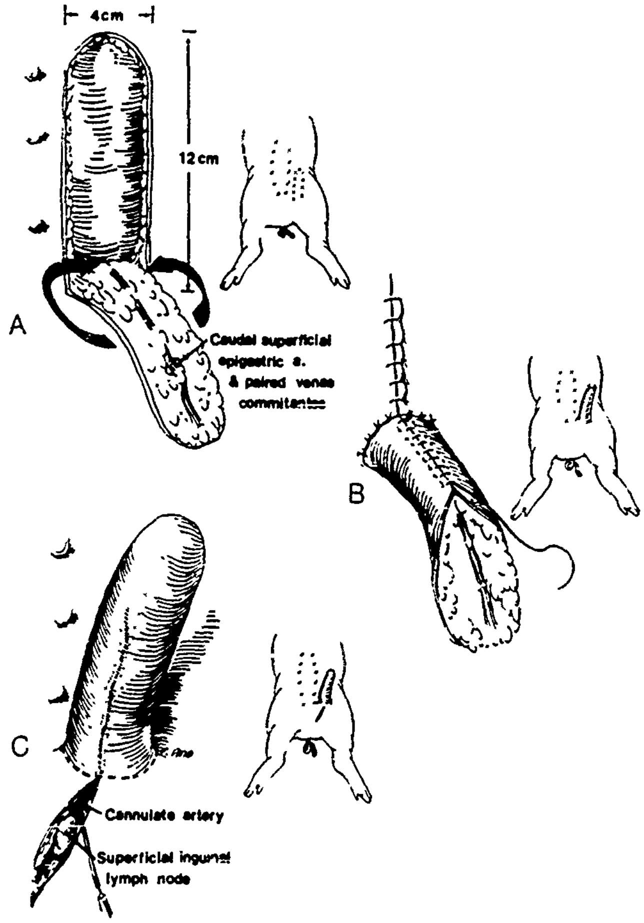

FIGURE 3: ROUTINE GENERAL ANESTHESIA IS ADMINISTERED TO 18-24 KG WEANLING YORKSHIRE PIGS AND THE CAUDAL ABDOMEN IS PREPARED FOR ASEPTIC SURGERY.

A - STAGE 1 PROCEDURE: INCISIONS ARE MADE ACCORDING TO THE LOCATION AND DIMENSIONS GIVEN AND DEEPENED TO THE LEVEL OF THE MUSCLE FASCIA. THE SKIN FLAP IS ELEVATED AND TRIMMED MINIMALLY OF FAT AT ITS EDGES.

B - THE TUBED FLAP IS FORMED AND ITS SKIN EDGES ARE CLOSED WITH NON-ABSORBABLE MONOFILAMENT SUTURE IN A SIMPLE CONTINUOUS PATTERN. THE DONOR SITE IS CLOSED PRIMARILY IN ROUTINE FASHION.

C - STAGE 2 PROCEDURE: THE TUBED FLAP IS RESECTED, ITS AXIAL ARTERY IS CANNULATED, AND THE TUBED FLAP IS TRANSFERRED TO THE IN VITRO ISOLATED PERFUSION APPARATUS.

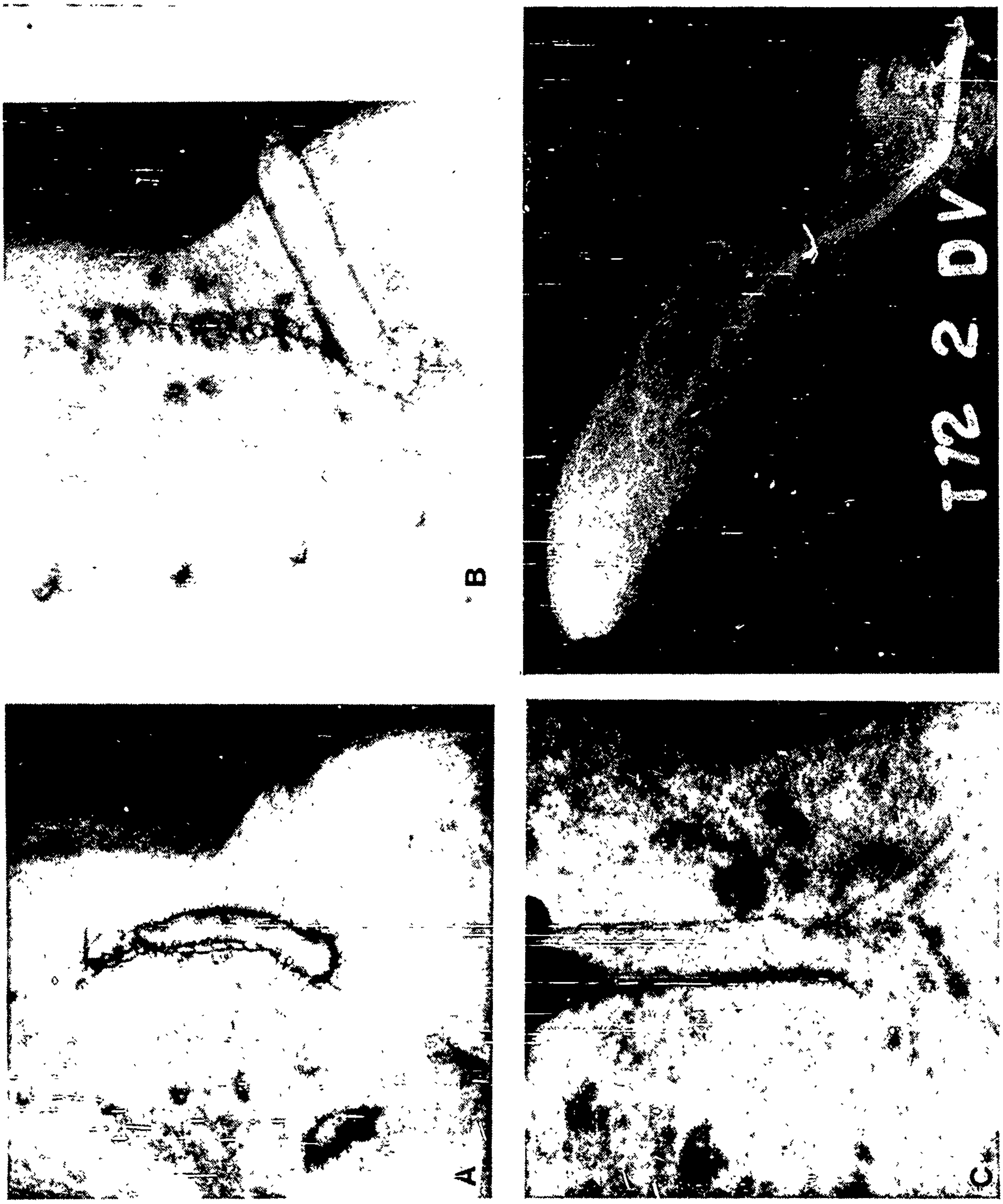
A
B
C
T12 2 DV

FIGURE 4: SINGLE PEDICLE, AXIAL PATTERN (ISLAND) TUBED SKIN FLAP IN PIG 21 (TUBE 12).

A - FLUORESCEIN ANGIOGRAPHY, A VIABILITY TEST FOR SKIN FLAP SURVIVAL, IMMEDIATELY FOLLOWING STAGE 1 PROCEDURE. BASED UPON OUR EXPERIENCE WITH THIS TECHNIQUE (N=52), THIS FLAP SHOULD SURVIVE TO ITS ENTIRE LENGTH.

B - SEVEN (7) DAYS POSTOPERATIVELY. NOTE SIMILAR APPEARANCE OF TUBED FLAP AND SURROUNDING SKIN AS WELL AS PLIABILITY OF TUBED FLAP. HEALING OF THE DONOR SITE IS EXCELLENT; PUNCTATE LESIONS SURROUNDING THE OPERATIVE SITE ARE SKIN REACTIONS TO STENT BANDAGE SUTURES.

C - TWENTY-EIGHT (28) DAYS POSTOPERATIVELY. NOTE HEALTHY APPEARANCE OF THE TUBED FLAP, ITS UNIFORM SIZE AND PLIABILITY, AND EXCELLENT HEALING OF INCISIONS.

D - MICROANGIOGRAPHY 28 DAYS POSTOPERATIVELY. NOTE COMPLETE FILLING OF THE AXIAL, SUBCUTANEOUS, AND DERMAL-SUBDERMAL VASCULATURE IN THIS TUBED FLAP.

CONCLUSIONS AND SUMMARY

WE HAVE DEVELOPED SURGICAL TECHNIQUES FOR PREPARATION OF IPPSF, CONSISTING OF A SINGLE PEDICLE, AXIAL PATTERN TUBED FLAP WHICH CAN: 1) BE RAISED IN 1 OPERATION; 2) SURVIVE TO ITS ENTIRE LENGTH; AND, 3) BE HARVESTED SUBSEQUENTLY FOR TRANSFER TO THE IN VITRO ISOLATED PERFUSION APPARATUS. OBVIOUSLY, CHARACTERIZATION OF THAT TUBED FLAP REGARDING ITS PHYSIOLOGICAL AND PHARMACOLOGICAL PROPERTIES DURING HEALING AND IN VITRO ISOLATED PERFUSION ARE NECESSARY. ALONE, THE STAGE 1 PROCEDURE MAY PROVIDE A UNIQUE IN SITU ISOLATED PERFUSION MODEL FOR STUDY OF PERCUTANEOUS ABSORPTION AND THESE STUDIES AND OTHERS ARE PLANNED TO BE CONDUCTED IN MID-1985. WHILE THE ADVANTAGES OF STUDYING PERCUTANEOUS ABSORPTION OR COUNTLESS OTHER PROBLEMS OF SKIN BIOLOGY, INCLUDING WOUND HEALING AND BURN PHENOMENA, IN THIS NOVEL IN VITRO MODEL ARE OBVIOUS, A HIDDEN BENEFIT IS ITS ACCEPTANCE AS A HUMANE, ALTERNATE ANIMAL MODEL. ONCE THE SKIN FLAP IS HARVESTED AND THE DONOR SITES HEAL COMPLETELY, THE PIG MAY BE RETURNED TO ITS PREVIOUS EXISTENCE.

THE AUTHORS THANK DRS. D.E. THRALL, S.W. CRANE AND L.P. DIX; AND, MS. LISA JACKSON FOR TECHNICAL ASSISTANCE. DR. R.L. CLARKE, DEPARTMENT OF RADIOLOGY, SCHOOL OF MEDICINE, UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL PROVIDED ASSISTANCE IN DEVELOPING THE MICROANGIOGRAPHY TECHNIQUES.

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HISTOLOGIC CHANGES CAUSED BY APPLICATION OF LEWISITE ANALOGS TO MOUSE SKIN AND HUMAN SKIN XENOGRAFTS

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ABSTRACT

Phenyldichloroarsine (PDA), a vesicating analog of lewisite, was applied in an ethanol carrier to human skin xenografts on nude mice and directly to the ungrafted nude mouse skin. Control areas received ethanol. The animals were killed from 30 min to 48 hr after application of 1.26 umoles PDA onto an area of 0.126 cm^2. Skin sections were examined by light and electron microscopy. Under light microscopy, we observed the following changes in PDA-treated human skin grafts: 1) epidermal cellular nuclear degeneration (apparent by 4 hr with increasing severity through 48 hr); 2) loss of epidermal cytoplasmic basophilia (apparent by 4 hr, maximum within 12 hr); 3) epidermal cytoplasmic vacuolization (vacuoles appeared within 4 hr and increased in size through 24 hr); 4) cleft formation within the basement membrane zone (apparent by 12 hr, increasing in severity through 24 hr); 5) inflammation [polymorphonuclear leukocyte (PMN) infiltration], apparent by 4 hr and increasing through 48 hr. The PMNs formed a wall around the lesion, but did not infiltrate the treated area. In nude mouse skin, the changes were similar and occurred more quickly, except for cytoplasmic vacuoles which were only occasionally observed. Nude mouse hair follicles and sebaceous glands showed similar cellular changes at approximately the same time as did epidermal cells. Transmission electron microscopy of mouse skin exposed to PDA up to 6 hr revealed a widening of intercellular spaces with attenuation of desmosomes. The subepidermal clefts resulted from separation within the lamina lucida of the basement membrane with the lamina densa forming the base of the cleft.

The following additional lewisite analogs were applied in ethanol carriers to nude mouse skin: phenylarsine oxide, phenyldiiodoarsine, (trans)chlorovinylarsine oxide and (trans)chlorovinyldiiodo arsine. The lesions caused by these analogs were reproducible and histologically indistinguishable from that caused by exposure to PDA. The identities of the molecular lesions and the locations of the arsenical-sensitive sites are unknown.

INTRODUCTION

The skin vesicating action of organic arsenicals is well known, yet their mechanism of action is poorly understood. The design of antidotes to vesicant arsenicals requires knowledge of their molecular mechanisms and sites of action. Because there is no ethically acceptable means of studying these effects on the skin of human volunteers, an *in vitro* system or animal model is needed. One such model, developed at LAIR, is human skin xenografts on the nude mouse. In this study we examined the morphological changes in nude mouse skin and in human skin xenografts on the nude mouse following application of phenyldichloroarsine (PDA) and other lewisite analogs with similar vesicant potency.

METHODS

PDA, a vesicating analog of lewisite, was applied in an ethanol carrier to human skin xenografts on nude mice and to the ungrafted nude mouse skin. Controls received only ethanol. Animals were killed at intervals of 30 minutes to 48 hours after application of 1.26 umoles PDA to an area of 0.126 cm^2. The following additional lewisite analogs were applied in ethanol carriers to nude mouse skin for 24 hours: phenylarsine oxide, phenyldiiodoarsine, (trans)chlorovinylarsine oxide, and (trans)chlorovinyldiiodide. Skin sections were examined by light and electron microscopy.

RESULTS

Figure 1. Untreated human skin xenograft on nude mouse. Epidermis (E) has the usual stratification of human skin, but rete ridges (R) are blunt or absent. The dermis (D) has lost its usual pattern and consists of undifferentiated fibroblastic proliferation with an increase of connective tissue fibers. Hematoxylin and eosin (H&E) x 70

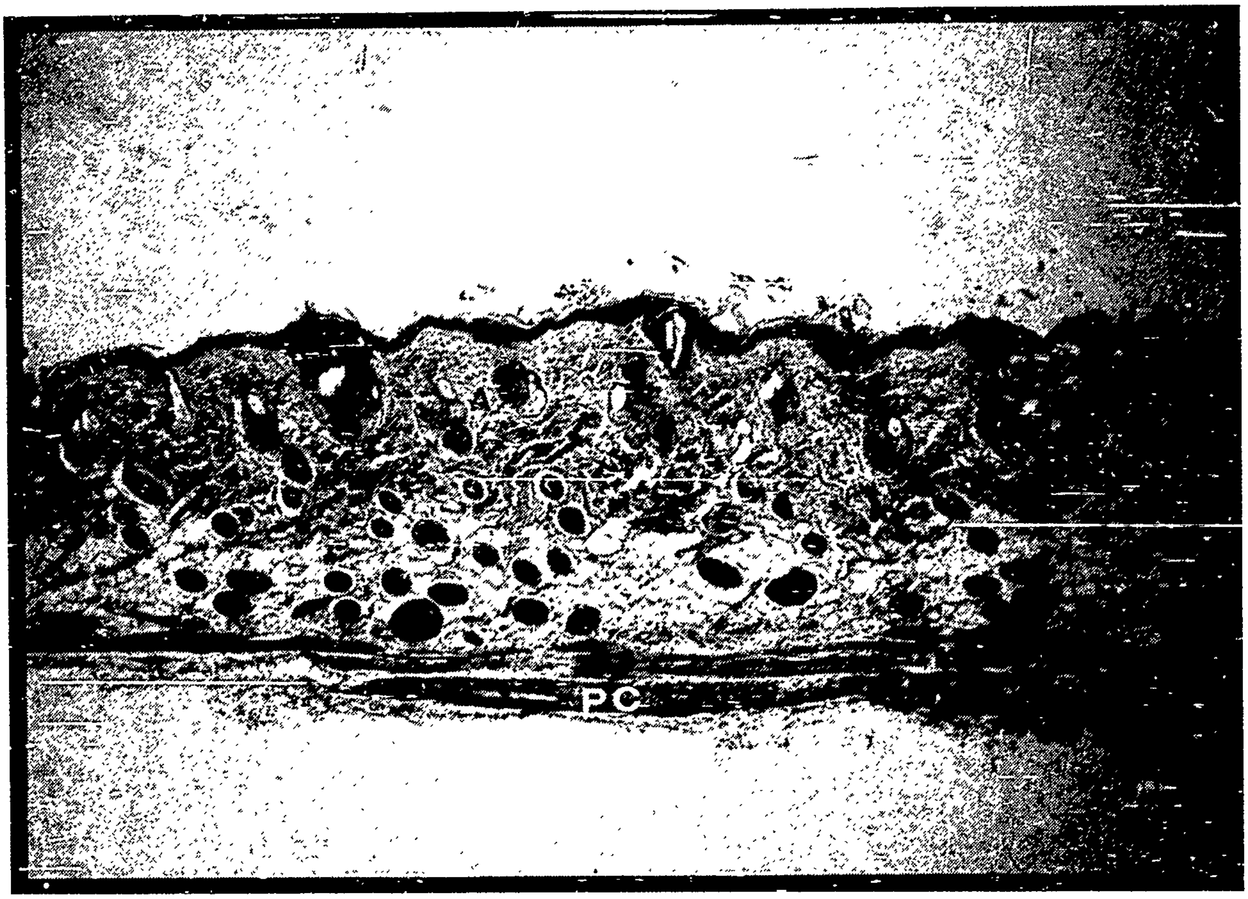

Figure 2. Untreated nude mouse skin. Note the relatively thin epidermal layer (E), abundance of adnexal structures (A), and thin dermal layer that is not differentiated into papillary and reticular layers as in human skin. The panniculus carnosus (PC), a thin layer of striated muscle, is separated from the dermis by a thin layer of adipose tissue. H&E × 70

Figure 3. Human skin xenograft 24 hr post exposure to PDA. A prominent zone of inflammatory cells (primarily polymorphonuclear leukocytes [PMN]) separates the area exposed to PDA from the adjacent untreated area. An increase in PMNs was first apparent at 4 hr and increased through 48 hr. Inflammatory cells do not infiltrate the PDA-treated area. There is severe damage to connective tissue fibers, adipose tissue, vessels and nerves in the subcutis. H&E x 70

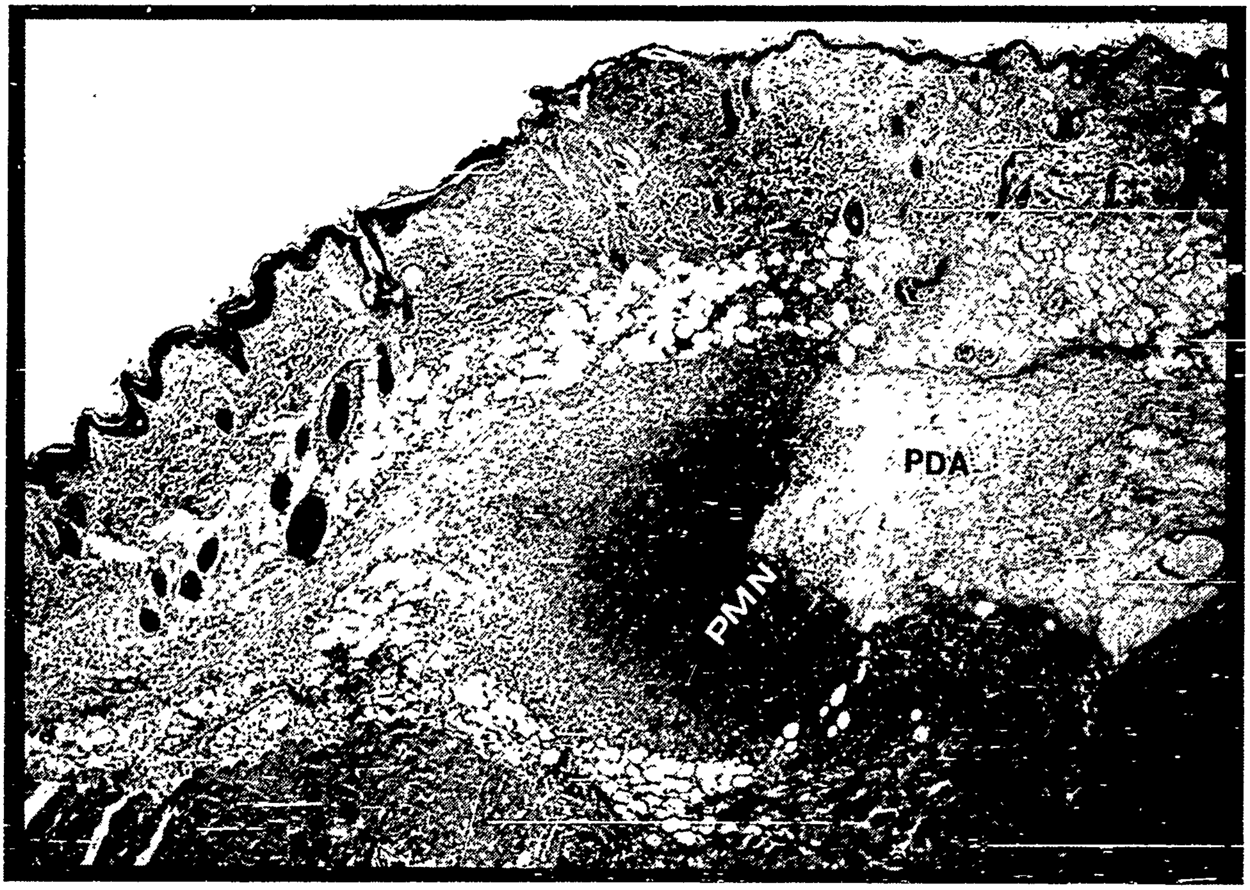

Figure 4. Nude mouse skin 24 hr post-exposure to PDA. A wall of polymorphonuclear leukocytes (PMN) separates area exposed to PDA from adjacent untreated area, but does not infiltrate the exposed area. There is severe damage to connective tissue, adipose tissue, muscle, vessels, and nerves in the subcutaneous area. H&E x 70

Figure 5. Human skin xenograft 24 hr after application of PDA. All layers of epidermis and dermis are undergoing degeneration and necrosis. Epidermal nuclear pyknosis was apparent by 4 hr and increased in severity through 48 hr. Loss of epidermal cytoplasmic basophilia was apparent by 4 hr and maximal at 12 hr. Vacuoles first appeared in epidermal cells at 4 hr and increased in size through 24 hr. Sub-epidermal clefts (C) first appeared at 12 hr and increased in severity through 24 hr. H&E x 280

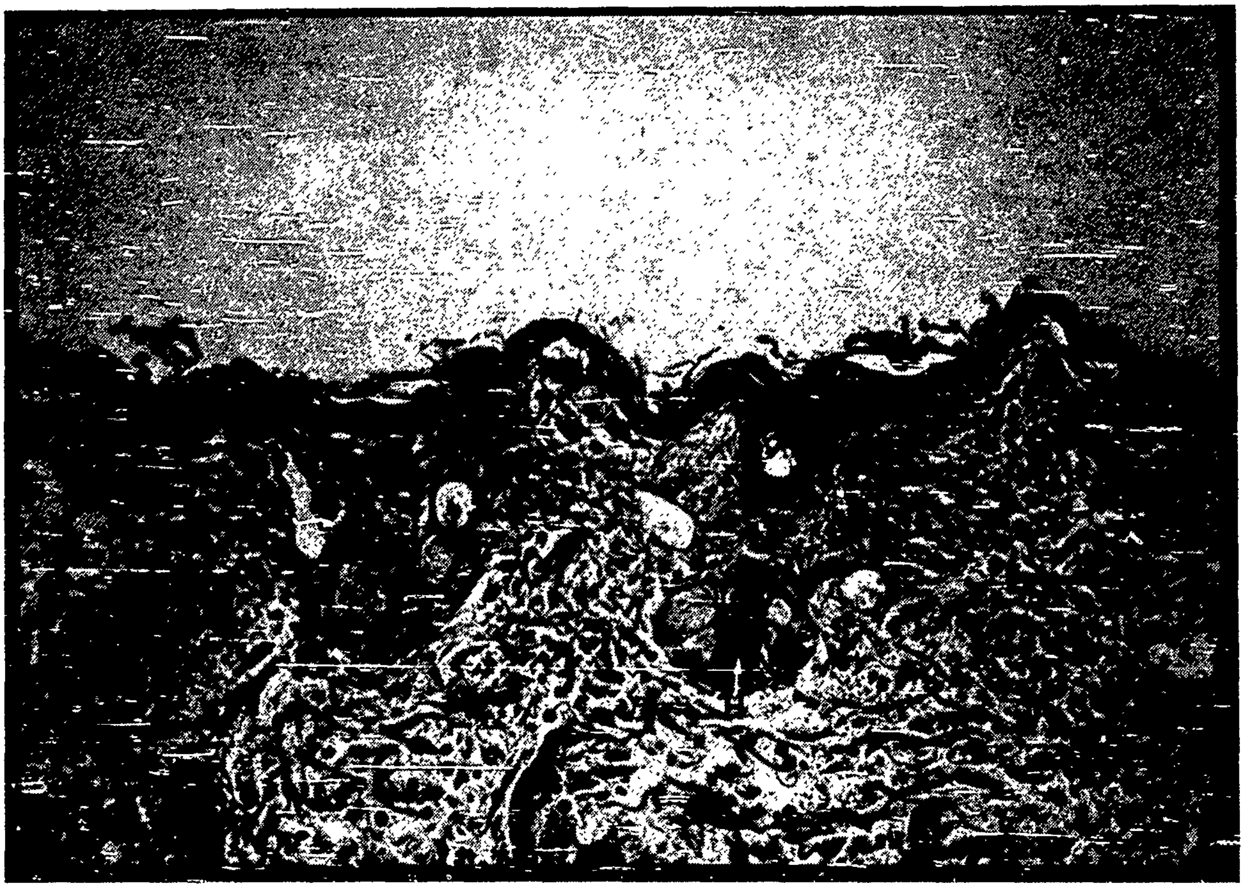

Figure 6. Nude mouse skin 24 hr after application of PDA. Changes are similar to those that occurred in human skin xenograft (see Fig. 5). In mouse skin, changes occurred more quickly than in human skin xenografts. Cytoplasmic vacuoles were only occasionally observed. Nude mouse hair follicles and sebaceous glands developed similar changes at approximately the same time as did epidermal cells. Dermal collagen fibers also have marked degenerative changes. H - hair follicle; S - sebaceous gland; C - sub-epidermal cleft. H&E x 280

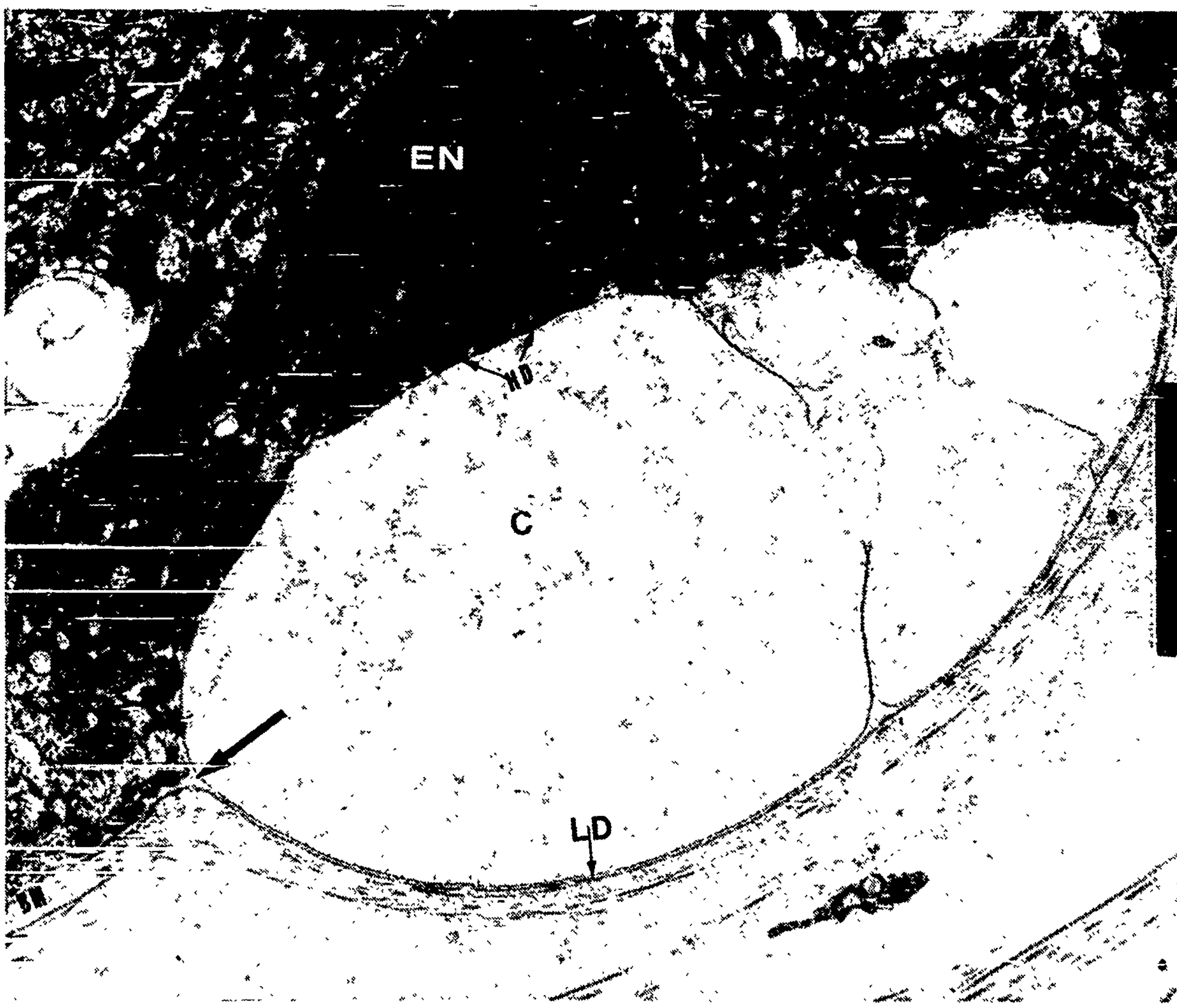

Figure 7. Electron micrograph of nude mouse skin 4 hr after PDA application. Sub-epidermal cleft (C) is formed by separation within the lamina lucida (arrow) of the basement membrane. The lamina densa (LD) forms the base of the cleft.
EN - epidermal cell nucleus; HD - hemidesmosome; BM - basement membrane
Uranyl acetate, lead citrate x 19,000

CONCLUSIONS

1. PDA, a vesicating analog of lewisite, causes reproducible histologic changes in both human skin xenografts and nude mouse skin.
2. Lesions in human skin xenografts and nude mouse skin are quite similar and occur at similar times after PDA application.
3. Four additional lewisite analogs, phenylarsine oxide, phenyldiiodoarsine, (trans)chlorovinylarsine oxide & (trans)chlorovinyldiiodo arsine produce lesions in nude mouse skin that are histologically indistinguishable from those caused by PDA.
4. Lewisite analogs cause separation of basement membrane lamina, an unusual ultrastructural lesion.
5. Molecular mechanisms of action and locations of arsenical-sensitive sites are unknown.

PATHOGENESIS OF THE DERMAL INFLAMMATORY RESPONSE TO SULFUR MUSTARD: MEDIATORS AND MODULATORS

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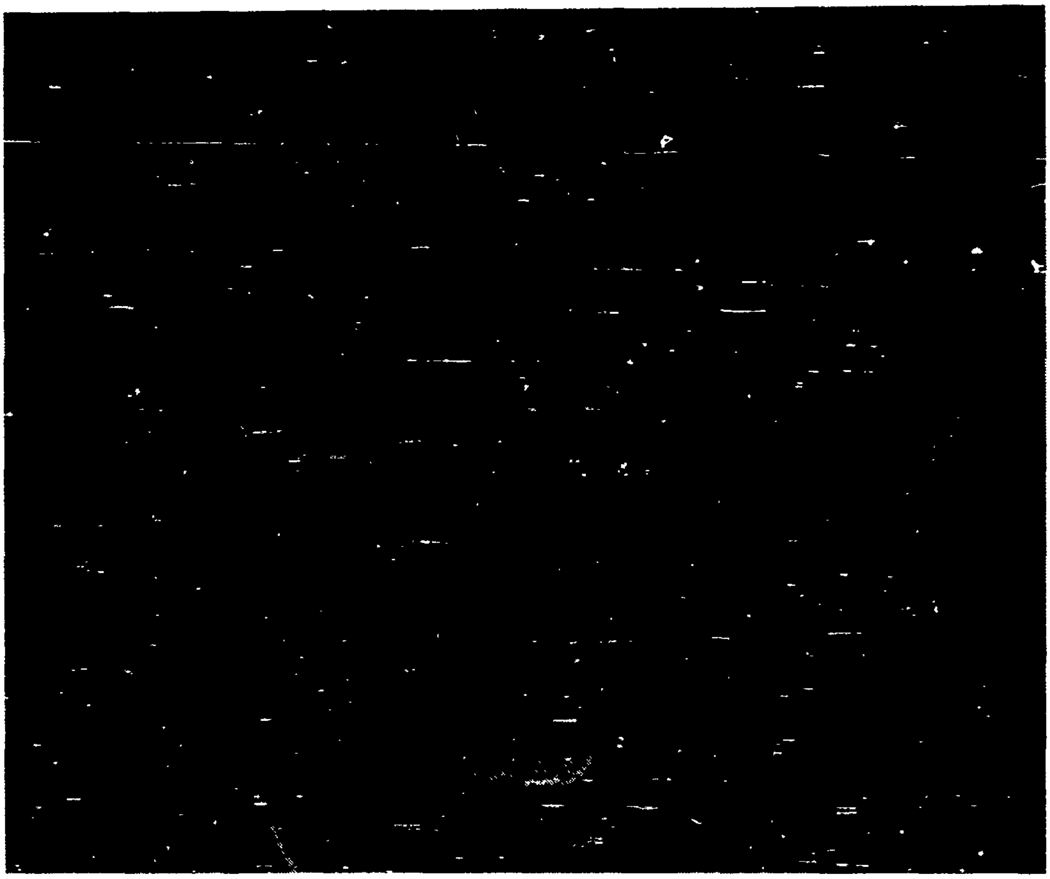

Figure 1. A 1.0 cm^2 explant of normal rabbit skin (above) and two 1-day sulfur mustard (SM) lesions (below) organ-cultured, for 3 days. By culturing developing and healing sulfur mustard (SM) lesions, we could collect various mediators and modulators of the inflammatory response and correlate the presence of these mediators with pathological alterations observed histologically.

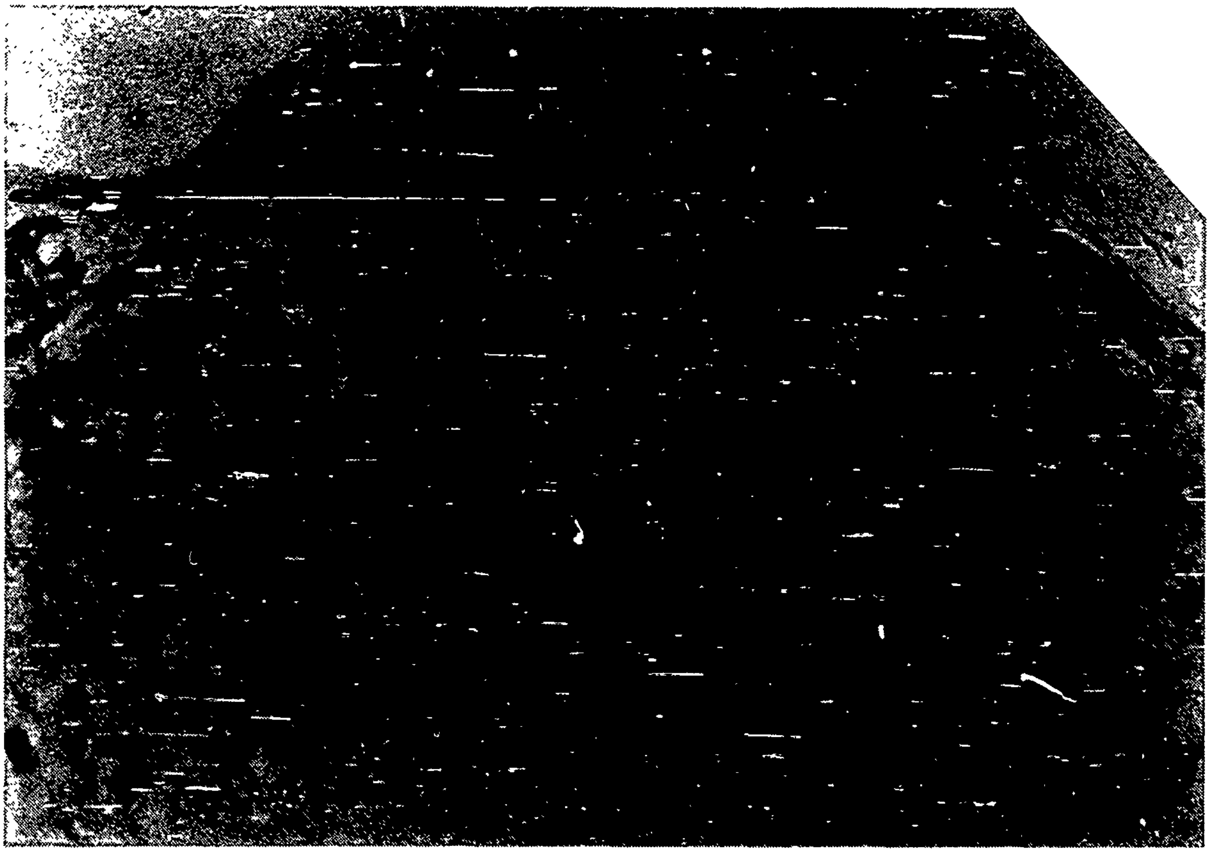

Figure 2. A 2 um glycol methacrylate tissue section of a 1-day SM les'on, stained with Giemsa. The epidermis is thin and ulcerated where the PMN are accumulating. During the next few days, these PMN die and form a crust that covers the ulcer bed. At 3 days, epithelial cells begin to migrate under the crust from the margins of the ulcer and from the uninjured hair follicles. By 10 days, healing is almost complete. X 1000.

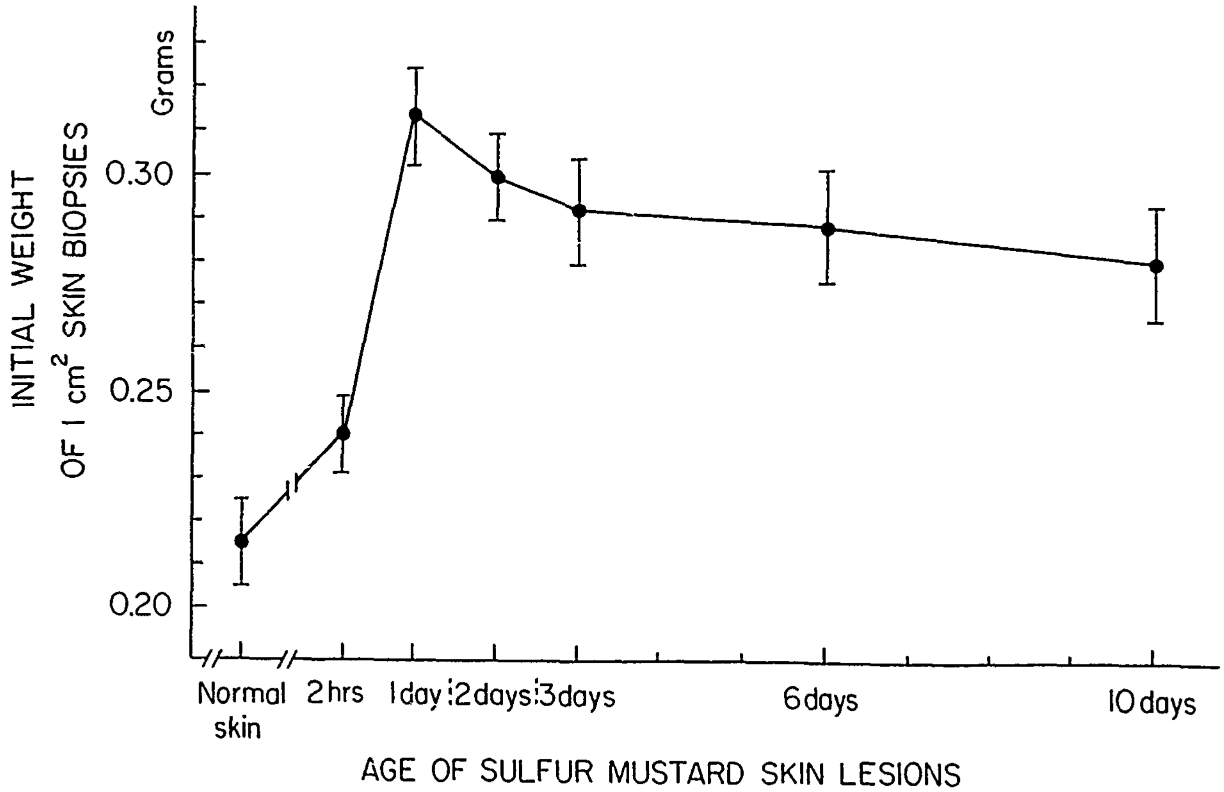

Figure 3. Weights of 1.0 cm^2 SM lesion biopsies before culture. The lesions weighed more than normal skin, mostly because of edema and partly because of cell infiltration.

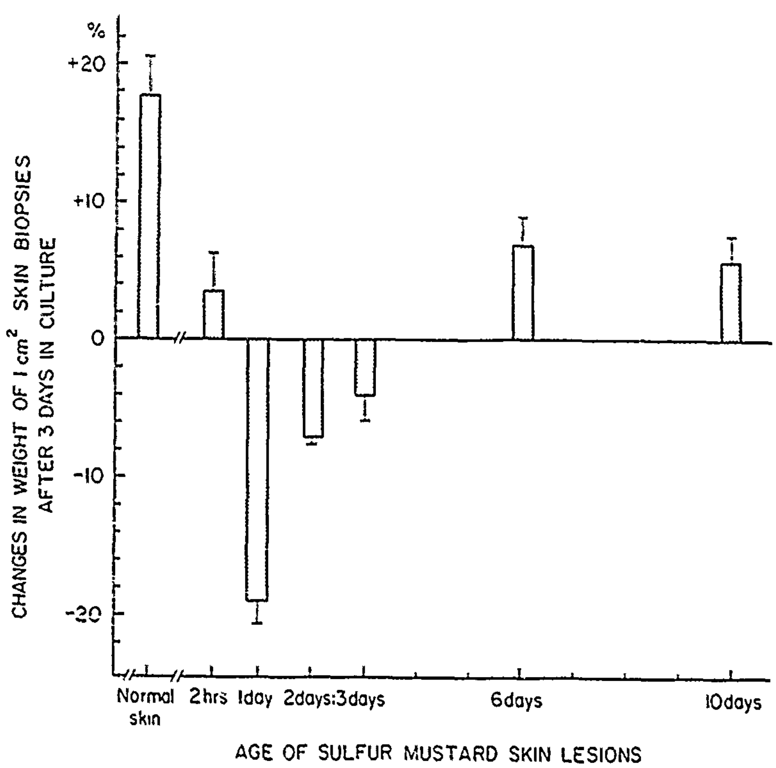

Figure 4. Changes in weight of lesion biopsies after 3 days in organ-culture. One-, 2- and 3 day lesions lost weight in culture; normal skin and healing lesions gained weight. These results can be explained by changes in the sol-gel state of the ground substance.

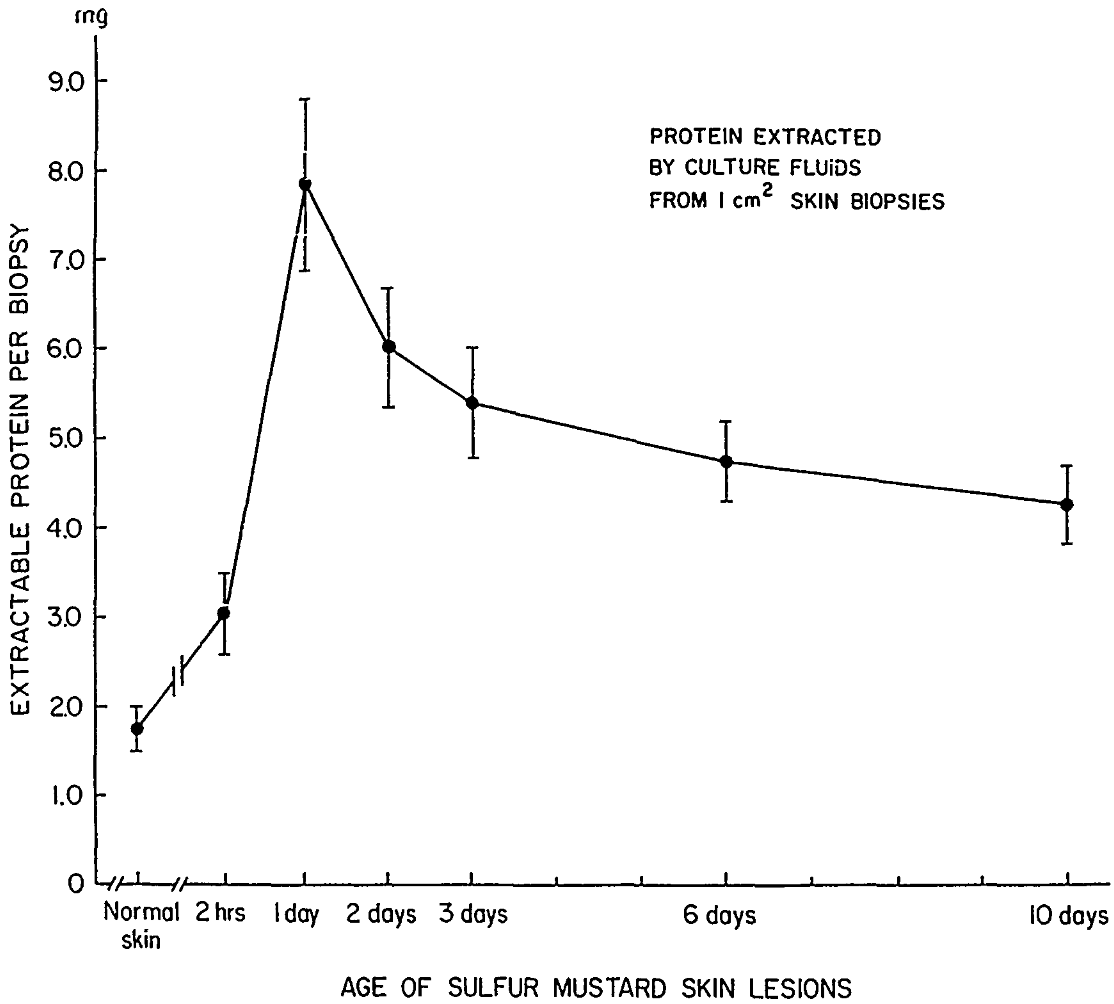

Figure 5. Protein extracted by the culture fluids. Both peak and healing lesions contained about 3 to 4 times the amount of such unbound protein as normal skin. This protein was mainly serum protein, which seems to be the major modulator of the inflammatory response.

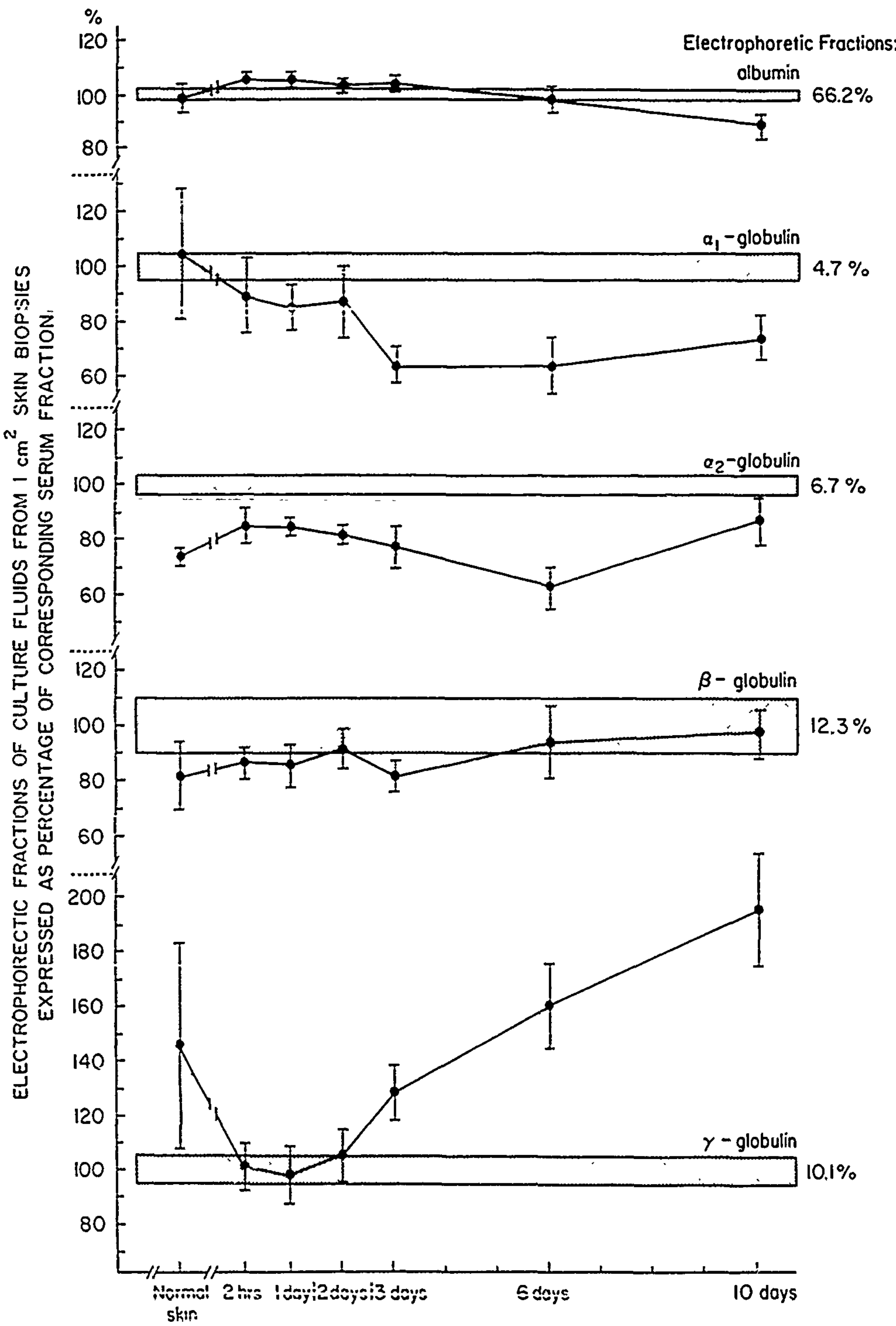

Figure 6. Electrophoretic fractions of the proteins in the culture fluids. In healing lesions, the a_1- and a_2-globulin fractions (containing protease inhibitors) were smaller and the gamma-globulin fraction (containing natural and specific antibodies) was larger than the corresponding fractions of serum. The antiproteases were reduced, because they probably formed complexes with leukocyte proteases.

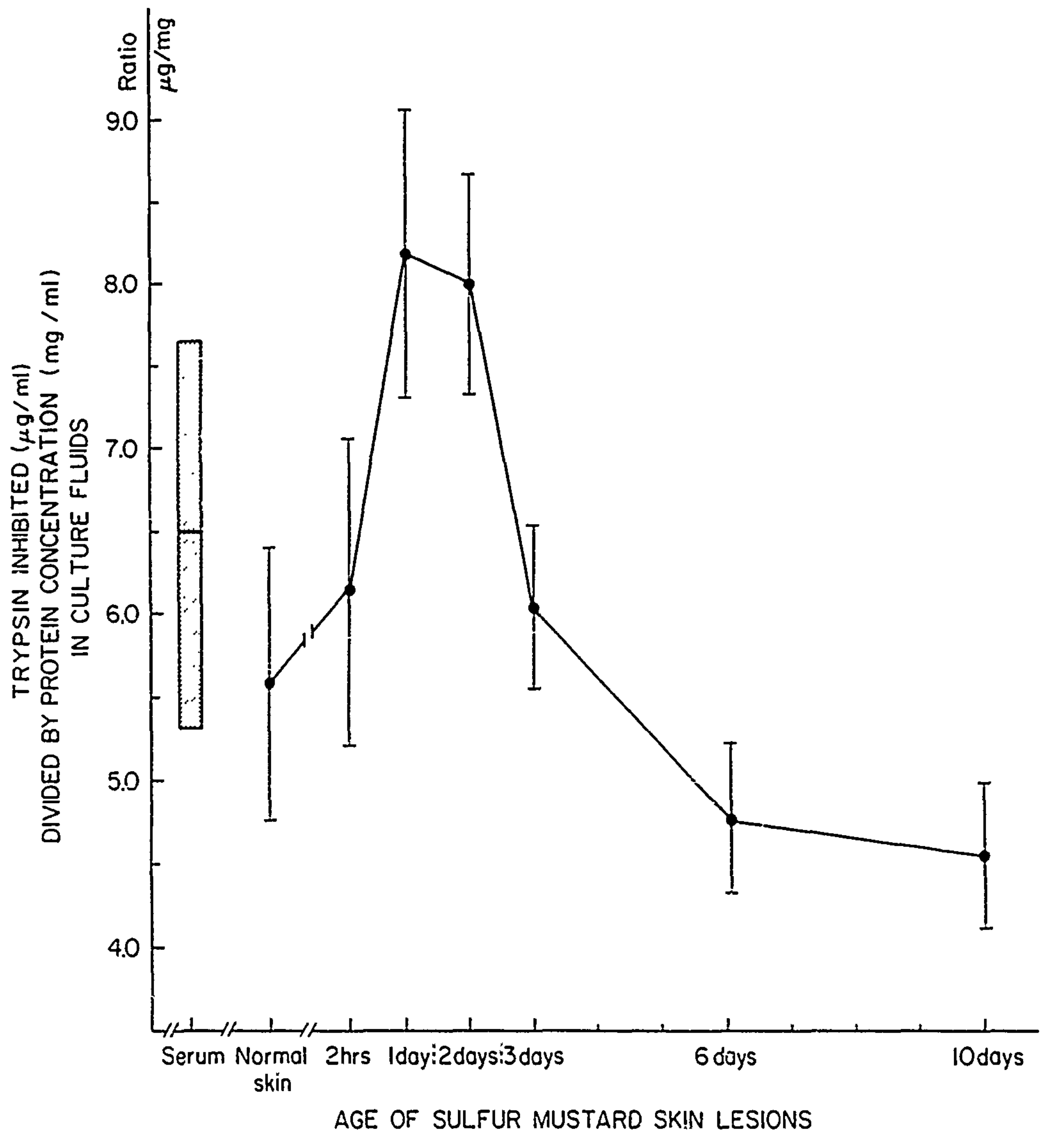

Figure 7. Trypsin inhibitory capacity of the culture fluids. This capacity was proportional to the (serum) protein content, except during healing (see Figure 6).

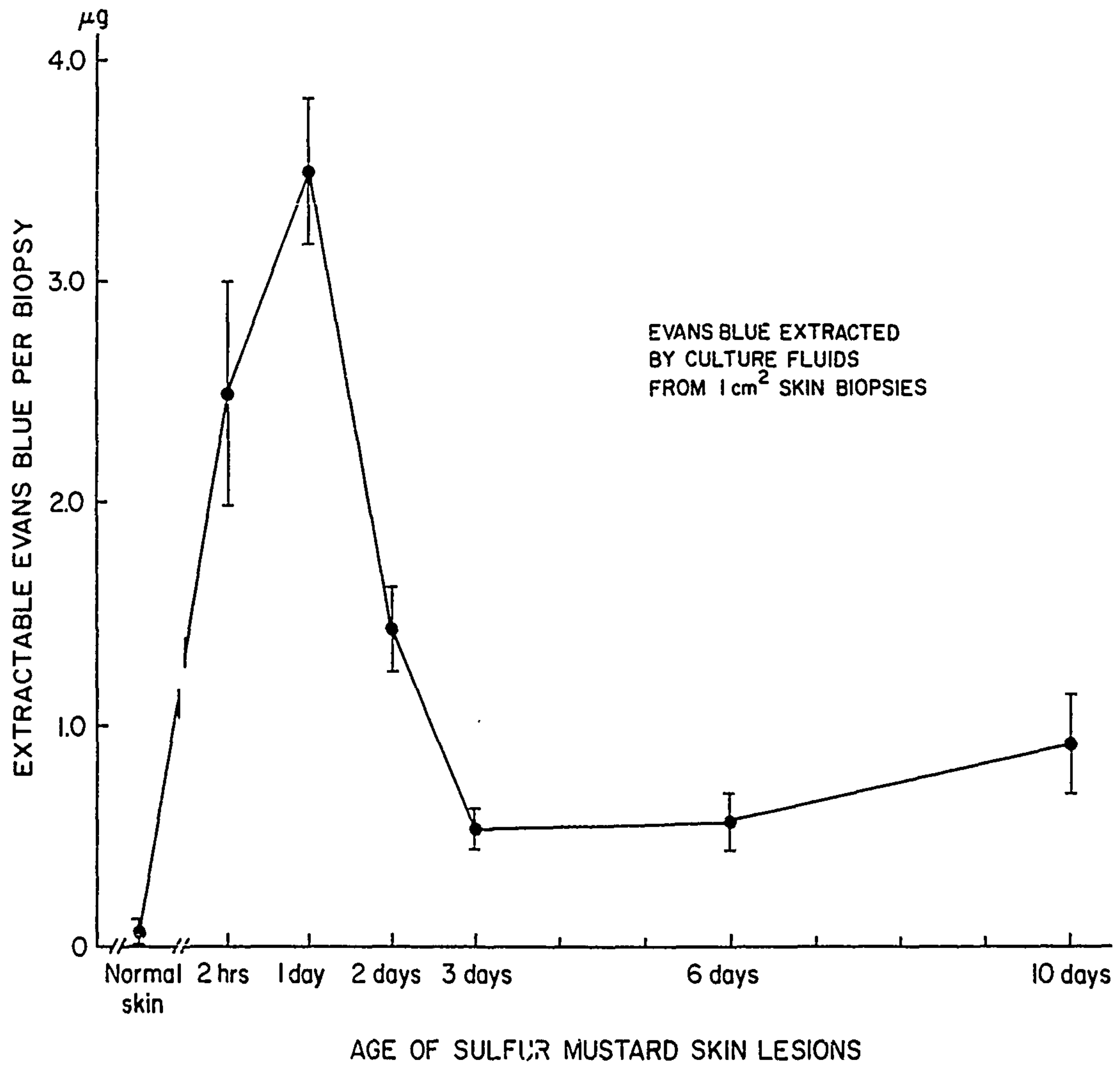

Figure 8 Evans blue extracted by the culture fluids from lesions of various ages. Evans blue (20 mg/kg) was injected intravenously 2 hours before the rabbit was sacrificed. It labeled serum protein, mainly albumin. This labeled serum protein extravasated into the lesions, especially during the first two days, but such extravasation decreased markedly as the lesions healed. A comparison of the total protein in the culture fluids (Figure 5) with the Evans blue-labeled protein (this Figure) enabled us to determine the turnover rates of unbound serum protein (albumin) in lesions of various ages (see Figure 9).

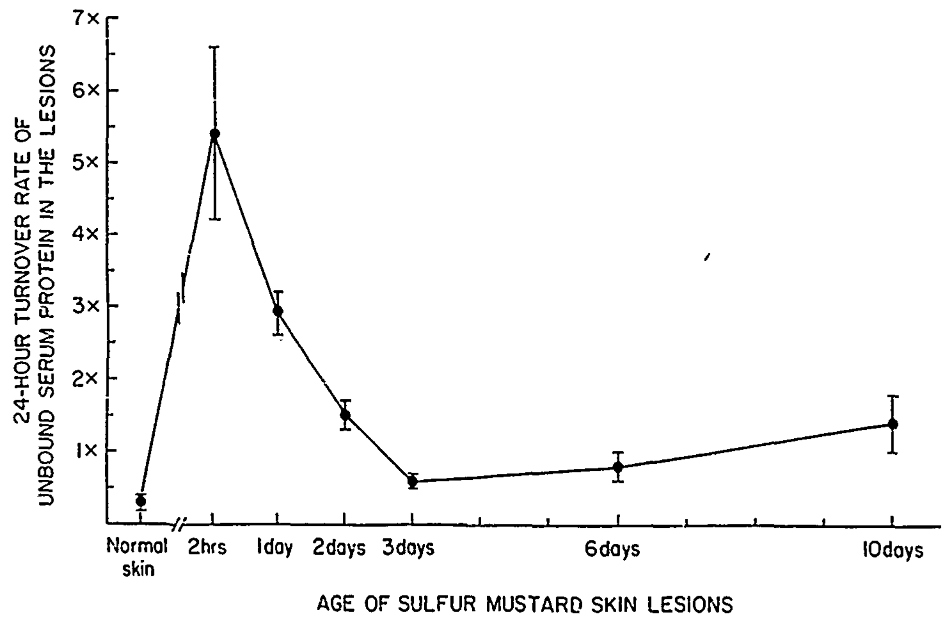

Figure 9. Daily turnover rates of unbound serum protein (albumin) in developing and healing lesions, determined as described in Figure 8. The bound serum protein (encapsulated within lymphatics) probably had similar turnover rates. These findings indicate that edema fluid is in a constant state of turnover and that it is not static, waiting to be absorbed.

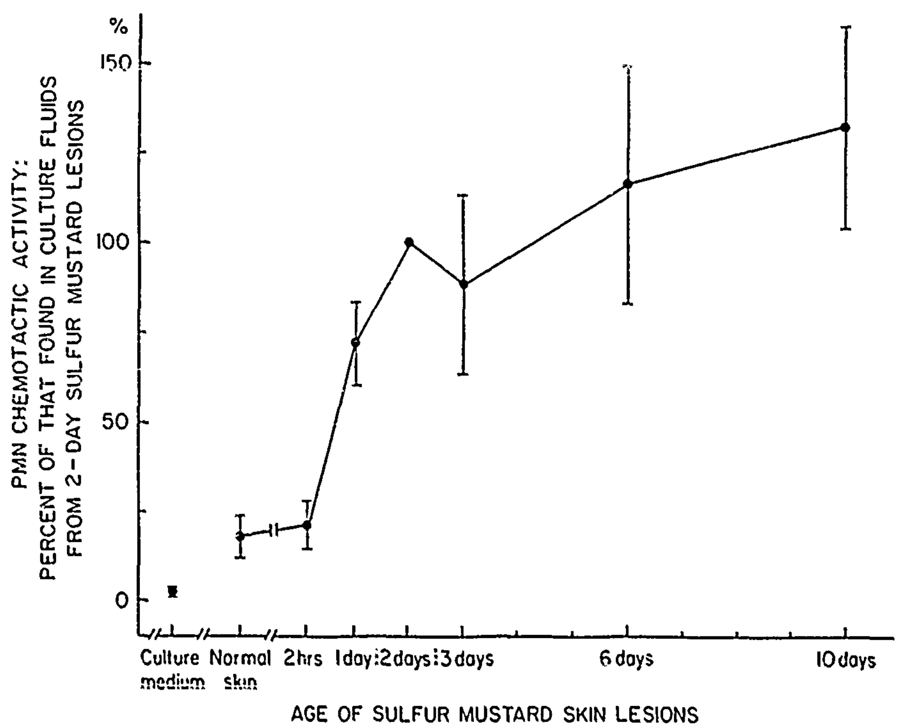

Figure 10. PMN chemotactic activities in the culture fluids. These activities remain high in lesions of all ages. Complement components (C5a) and leukotriene B_4 are being evaluated as the source of the chemotactic activity. Monocyte chemotactic activity was also increased.

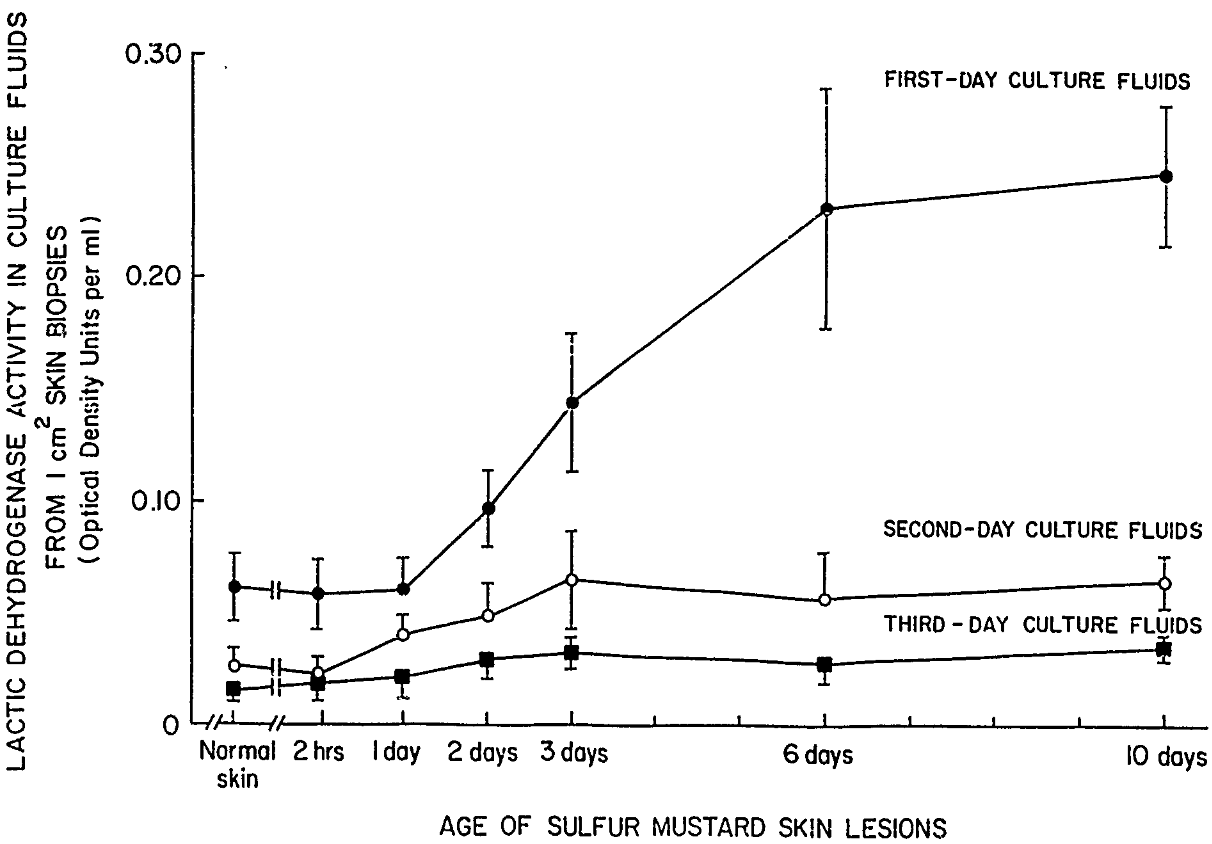

Figure 11. Lactic dehydrogenase (LDH) activities in the culture fluids. Various hydrolytic enzymes, including chymotrypsin-like esterase, proteoglycanase and collagenase, were increased as the lesions healed. Live and dead PMN, macrophages and fibroblasts were probably the source of these enzymes.

SUMMARY

The skin lesion produced by sulfur mustard (SM) is an ideal model in which to study slowly developing acute inflammation. SM, a DNA alkylating agent, gradually kills epidermal cells and causes generalized vascular leakage, leukocyte infiltration, blistering (in man), and ulceration.

Developing and healing SM lesions were organ-cultured, and the culture fluids were assayed for the various mediators and modulators of the inflammatory process. Extravasated serum seemed to provide most of the mediators and modulators of the early inflammatory response; whereas dying PMN, activated macrophages and activated fibroblasts seemed to provide most of the mediators and modulators associated with healing.

SIGNIFICANCE

Our studies disprove the concept that extravasted serum in sites of injury is static, waiting to be absorbed. Unexpectedly, such serum had a high local turnover rate, even when the SM lesions were at their peak. This serum seems to play a major beneficial role: Serum chemotaxis inhibitors prevent the excess infiltration of leukocytes. Serum antiproteases protect against the destruction by proteases (from infiltrating leukocytes). Serum ceruloplasmin protects against oxidant damage (again from leukocytes). Finally, serum transports therapeutic agents into the exact place where they are needed.

MODIFICATION OF DNA BY CHLOROETHYL ETHYL SULFIDE AND ITS REPAIR BY RAT LIVER EXTRACTS

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INTRODUCTION

EARLIER STUDIES HAVE ATTRIBUTED THE CYTOTOXIC EFFECTS OF SULFUR MUSTARD (BIS-CHLOROETHYL SULFIDE) TO THIS AGENT'S ABILITY TO CROSSLINK DNA THROUGH THE 7 POSITION OF GUANINE. HOWEVER, SEVERAL INVESTIGATORS HAVE SHOWN THAT MONOFUNCTIONAL SULFUR MUSTARDS ARE LETHAL TO BACTERIA AND CELLS IN CULTURE, INDICATING THAT MONOFUNCTIONAL LESIONS ARE LETHAL AS WELL. FURTHERMORE, GENETIC DATA SUGGEST THAT OTHER DNA MODIFICATIONS INCLUDING ALKYLATION OF THE 6 POSITION OF GUANINE MAY BE RESPONSIBLE FOR MUTAGENICITY AND CARCINOGENICITY.

IT IS NOW KNOWN THAT MOST CELLS HAVE THE ABILITY TO REPAIR DNA LESIONS, SO THAT SOME NATURAL PROTECTION AGAINST THESE AGENTS IS POTENTIALLY AVAILABLE.

WE HAVE UNDERTAKEN A STUDY TO DETERMINE WHAT DNA LESIONS ARE PRODUCED BY A TYPICAL MONOFUNCTIONAL MUSTARD, CHLOROETHYL ETHYL SULFIDE (CEES), AND TO ASCERTAIN WHICH OF THESE LESIONS ARE REPAIRED BY MAMMALIAN REPAIR FACTORS. THE LONG-RANGE OBJECTIVE OF THESE STUDIES IS TO PROVIDE PROTECTION AGAINST THE SULFUR MUSTARDS BY INDUCING AN INCREASED LEVEL OF REPAIR FACTORS OR BY SYNTHESIZING THESE FACTORS SO THAT THEY COULD BE ADMINISTERED EXOGENOUSLY.

METHODS

1. Modification of DNA with ^{14}C-CEES

DNA (0.8 mg/ml) was incubated for 1 hr in neutral buffer at 37° with ^{14}C-CEES (0.2 mM) to provide a suitably modified substrate for repair studies. Unbound ^{14}C-CEES residues were removed by repeated reprecipitations with ethanol.

2. Analysis of DNA Modifications

DNA was digested to the deoxynucleoside level with a combination of nucleases and alkaline phosphatase. Digests containing approximately 0.2 mg (20,000 cpm) were separated by HPLC on a C_{18} column eluted with 14% acetonitrile in 25 mM KH_2PO_4, pH 4.5, at 1 ml/min. 0.5-min fractions were collected and analyzed for radioactivity.

3. Preparation of Mammalian Repair Enzymes

Livers from partially hepatectomized rats were homogenized and cellular debris was removed by centrifugation. DNA repair enzymes were partially purified by ammonium sulfate fractionation and by use of a DNA-cellulose column.

4. DNA REPAIR STUDIES

APPROXIMATELY 0.2 MG OF ^{14}C-CEES-MODIFIED SUBSTRATE DNA WAS INCUBATED WITH VARYING AMOUNTS OF REPAIR PROTEINS. CONTROL TUBES CONTAINED THE SAME AMOUNT OF DNA AND HEAT-INACTIVATED REPAIR PROTEIN OR ALBUMIN. AFTER 1 HR'S INCUBATION AT 37°, THE DNA WAS DIGESTED AND ANALYZED AS DESCRIBED ABOVE. NOTE THAT THIS ASSAY METHOD IS DESIGNED TO REVEAL REPAIR BY THE ALKYLTRANSFERASE METHOD, BUT WILL NOT DEMONSTRATE GLYCOSYLASE ACTIVITY.

5. ANALYSIS OF DATA

HPLC PROFILES OF DIGESTED DNA WERE COMPUTER ANALYZED INTO THEIR CONSTITUENT NUCLEOSIDE COMPONENTS. EXPERIMENTAL AND CONTROL TUBES WERE THEN COMPARED TO DETERMINE WHETHER REPAIR BY THE ALKYLTRANSFERASE MECHANISM HAD OCCURRED.

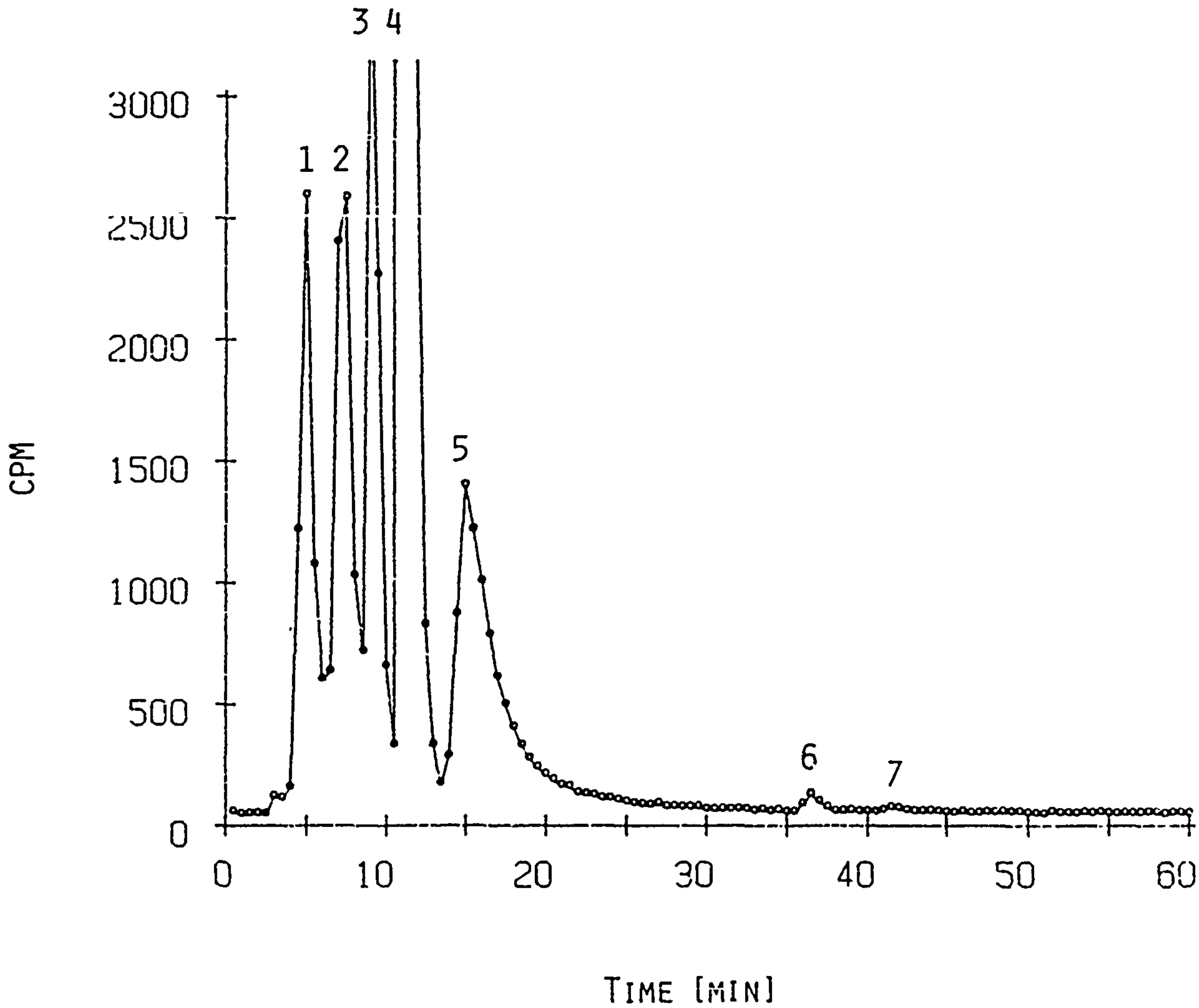
RESULTS

1. ANALYSIS OF ^{14}C-CEES-MODIFIED DNA REVEALS THE PRESENCE OF 7 DISTINCT DERIVATIVE PEAKS. THE MAJOR DERIVATIVE IS N^7-ETHYLTHIOETHYL GUANINE AND THE SECOND-LARGEST DERIVATIVE IS N^3-ETHYLTHIOETHYL ADENINE, IN AGREEMENT WITH PREVIOUS FINDINGS. HOWEVER, SEVERAL ADDITIONAL UNKNOWN PEAKS ARE PRESENT AND TOGETHER THESE CONSTITUTE 25% OF THE DNA MODIFICATION. SEVERAL OF THESE ARE ACID LABILE WHICH HELPS TO EXPLAIN WHY THEY WEREN'T DETECTED IN EARLIER STUDIES WHICH ALSO DID NOT USE HPLC TECHNOLOGY.

2. O^6-ETHYLTHIOETHYL DEOXYGUANOSINE, ALTHOUGH PRESENT IN SMALL AMOUNTS, COULD HAVE CONSIDERABLE BIOLOGICAL SIGNIFICANCE. STUDIES OF RAT LIVER EXTRACT WHICH HAVE THE CAPACITY TO REPAIR O^6-METHYL DEOXYGUANOSINE IN DNA HAVE LITTLE OR NO CAPACITY TO REPAIR O^6-ETHYLTHIOETHYL DEOXYGUANOSINE.

3. THESE SAME RAT LIVER EXTRACTS DO HAVE THE CAPACITY TO REPAIR ONE OF THE UNIDENTIFIED DERIVATIVE PEAKS IN DNA.

HPLC ANALYSIS OF DNA MODIFIED WITH ^{14}C-CEES



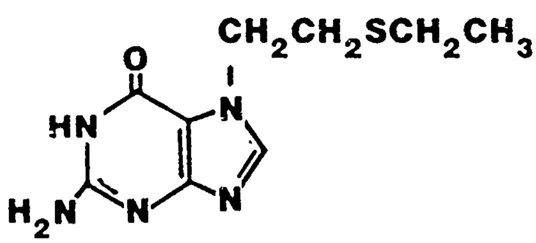
DNA MODIFIED WITH ^{14}C-CEES WAS DIGESTED TO THE NUCLEOSIDE LEVEL AND ANALYZED AS DESCRIBED ABOVE. THE COMPOSITION OF THIS DNA IS GIVEN IN TABLE 1.

TABLE 1

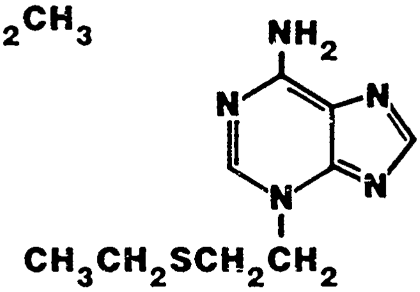
HPLC ANALYSIS OF ^{14}C-CEES-MODIFIED DNA

PEAK #	IDENTITY	PERCENT
1	NOT YET IDENTIFIED	7.0
2	NOT YET IDENTIFIED	9.0
3	NOT YET IDENTIFIED	9.0
4	7-ETHYL-S-ETHYL GUANINE	63.0
5	3-ETHYL-S-ETHYL ADENINE	12.0
6	NOT YET IDENTIFIED	0.3
7	O^6-ETHYL-S-ETHYL DEOXYGUANOSINE	0.1

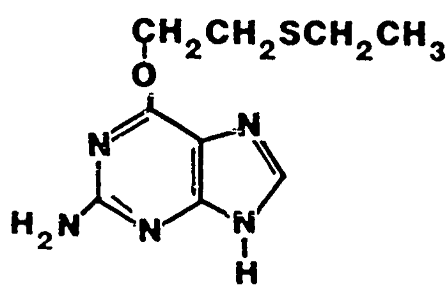
MODIFICATION OF DNA BY CEES



7-ethylthioethyl guanine



3-ethylthioethyl adenine



O^6-ethylthioethyl guanine

STRUCTURES OF BASES MODIFIED BY CEES. PEAK 4 IN THE ACCOMPANYING HPLC ANALYSIS IS 7-ETHYLTHIOETHYL GUANINE; PEAK 5 IS 3-ETHYLTHIOETHYL ADENINE; AND PEAK 7 IS O^6-ETHYLTHIOETHYL DEOXYGUANOSINE (THE DEOXY-NUCLEOSIDE OF O^6-ETHYLTHIOETHYL GUANINE).

TABLE 2

REPAIR OF PEAK 3 BY RAT LIVER EXTRACT

EXPERIMENT #	ENZYME ADDED (UNITS/PMOLE)*	DERIVATIVE IN DNA (PMOLE/MG)		PERCENT CHANGE
		CONTROL	EXPERIMENTAL	
547	0.004	632	597	6
549	0.013	521	384	26
564	0.013	1269	878	31
551	0.025	582	412	29
559	0.029	611	321	48
561	0.050	506	191	62

* 1 UNIT OF ACTIVITY WILL REPAIR 1 PMOLE O^6-METHYL GUANINE IN DNA.

TABLE 3

LACK OF REPAIR OF O^6-ETHYLTHIOETHYL GUANINE IN DNA

EXPERIMENT #	ENZYME ADDED (UNITS/PMOLE)*	DERIVATIVE IN DNA (PMOLE/MG)		PERCENT CHANGE
		CONTROL	EXPERIMENTAL	
547	0.9	3	6	
549	2.8	2	4	NO
564	2.8	3	6	SIGNIF.
551	5.6	3	2	DIFF.
559	6.5	4	3	
561	11.2	4	5	

* 1 UNIT OF ACTIVITY WILL REPAIR 1 PMOLE O^6-METHYL GUANINE IN DNA.

CONCLUSIONS

1. ANALYSIS OF DNA MODIFIED BY ^{14}C-CEES REVEALS THAT THE CHEMISTRY OF DNA MODIFICATION IS MORE COMPLEX THAN PREVIOUSLY APPRECIATED FOR EVEN THIS ONE-ARMED SULFUR MUSTARD.

2. WE HAVE SHOWN THAT CEES MODIFIES DNA IN THE O^6 POSITION OF GUANINE AS PREVIOUSLY POSTULATED BUT NOT PREVIOUSLY DEMONSTRATED. THIS LESION APPEARS TO BE RESISTANT TO REPAIR BY A PROTECTIVE ENZYME FOUND IN MAMMALIAN CELL EXTRACTS WHICH CAN REMOVE A METHYL GROUP FROM THE O^6 POSITION OF GUANINE.

3. THESE SAME CELLULAR EXTRACTS HAVE THE ABILITY TO REPAIR ANOTHER, STILL-UNIDENTIFIED DERIVATIVE PEAK IN DNA.

4. SINCE THE ASSAY USED IN THESE STUDIES IS DESIGNED TO DEMONSTRATE REPAIR BY THE TRANSFERASE MECHANISM ONLY, OTHER MECHANISMS MAY BE INVOLVED IN THE REPAIR OF OTHER DNA MODIFICATIONS REVEALED BY THESE STUDIES. THESE REPAIR MECHANISMS MAY PROVIDE PROTECTION AGAINST THE ACUTE AND DELAYED TOXICITY OF THE SULFUR MUSTARDS.

MODIFIED CLAYS AS POTENTIAL DECONTAMINATION AGENTS FOR SKIN

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OBJECTIVES

- Modify natural clays to increase the adsorption and/or reactivity of the powder
- Select simulants for blister agent (mustard) and nerve agents (soman and VX)
- Develop a test procedure to measure adsorption and/or reaction of clay with agent or simulant
- Develop a test procedure to measure decomposition of agent or simulant by clay
- Evaluate a series of modified clays using these analytical procedures with the selected simulants

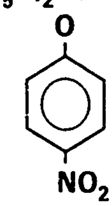
CLAY MODIFICATIONS

1. Clays with amines bound to the surface
2. Beneficiated clay minerals
3. High surface area—large pore minerals
4. Clays with intercalated metal ions
5. Acid activated clay minerals
6. Oxidative clay minerals

PROTOTYPES OF MODIFIED CLAYS ALREADY EVALUATION

1. Bentonite and Irwin clays with amines attached
2. Copper-treated clays from Item 1
3. Bentonite clay with amines intercalated
4. Clays with metal ions intercalated
5. Clays which were acid washed
6. Beneficiated clays which were calcined
7. Clays with high surface area

SIMULANTS FOR CHEMICAL WARFARE AGENTS

SIMULANT	STRUCTURE	ABBREV-IATION	AGENT SIMULANT	TYPE
CHLOROETHYL ETHYL SULFIDE	$ClCH_2CH_2SCH_2CH_3$	CEES	HD	BLISTER AGENT
DIETHYL CHLORO-PHOSPHATE	$(C_2H_5O)_2POCl$	DECP	GB	NERVE AGENT
DIETHYL *p*-NITRO-PHENYL PHOSPHATE	$(C_2H_5O)_2PO$ 	DNPP	GB	NERVE AGENT
O-ETHYL-*S*-ETHYL METHYLPHOS-PHONOTHIOATE	$(C_2H_5O)(C_2H_5S)P(=O)CH_3$	EEMPT	VX	NERVE AGENT
DIISOPROPYL FLUOROPHOSPHATE	$[(CH_3)_2CHO]_2P(=O){-}F$	DFP	GB	NERVE AGENT

ANALYTICAL METHODS

EVALUATION METHODS

Estimate of adsorption and decomposition of simulants

- Headspace Analysis—Vapor density of simulant above clay sample
- Washer Test—Breakthrough time of clay plug
- Wicking Test—Rate of simulant sorbed by powder sample (see Poster 102, "In Vitro Tests to Compare Skin Decontamination Powders" by M. M. Mershon et al.)

Estimate of decomposition and/or irreversible adsorption of simulant

- Exposure followed by ether extraction after 6 minutes
- Exposure followed by ether extraction after variable exposure times
- Exposure of variable amounts of simulant followed by ether extraction after 4 minutes

HEADSPACE ANALYSIS

1. A 200-mg sample of the modified clay or Fuller's Earth was placed in a 4-mL septum-sealed vial.
2. Using a microliter syringe, 10-20 μL portions of simulants were injected directly onto the clay and the vial was stirred using a Vortex mixer for 30 seconds.
3. A sample of vapor from the headspace was withdrawn using a 50-μL gas-tight syringe and analyzed by GC using the conditions given below.
4. Additional portions of simulants were added until the headspace gas contains the same amount of simulant as a vial containing simulant without any clay.

Typical results are shown in Figure 1. The significance of this method was not clear and did not give consistent results. The method was not applicable to nonvolatile simulants.

WASHER TEST

1. A washer of 10-mm inside diameter and thickness of 1.5 mm is placed on a sheet of VGH ABC-M-8 test paper.
2. The test sample is placed in the center of the washer and packed with a microscope slide.
3. The clay is spiked with 10 μL of simulant. After 4 minutes, if the paper shows no change, a second 10 μL spike is added. This process is repeated until a change in the color of the paper is noted.

Typical results are given in Table 1.

ANALYTICAL PARAMETERS FOR SIMULANT ANALYSIS

PHOSPHATE ESTER SIMULANT DECP

COLUMN	8' x 2 mm ID 3% OV-1 ON SUPELCOPORT (100/120 MESH)
OVEN TEMPERATURE	125° ISOTHERMAL
DETECTOR TEMPERATURE	250°
INJECTOR TEMPERATURE	150°
CARRIER/FLOW RATE	He/40 mL/MIN
ANALYTE RETENTION TIME	1.4 MIN
DETECTOR	ALKALI BEAD FLAME IONIZATION DETECTOR

ANALYTICAL PARAMETERS FOR SIMULANT ANALYSIS

MUSTARD SIMULANT CEES

COLUMN	6' x 4 mm ID 5% OV-1 ON CHROMOSORB W
OVEN TEMPERATURE	70° ISOTHERMAL
DETECTOR TEMPERATURE	250°
INJECTOR TEMPERATURE	250°
CARRIER/FLOW RATE	He/40 mL/MIN
ANALYTE RETENTION TIME	1.2 MIN
DETECTOR	FLAME IONIZATION

PROCEDURE FOR TESTING DECOMPOSITION OF SIMULANT BY CLAYS

1. Place 200 mg of clay into 4.0 mL vial.
2. Spike with 5.0 μL of 20% simulant solution (ether) or 5 μL neat simulant.
3. Mix contents of vial thoroughly (Vortex mixer 1.0 minute).
4. Allow contents to settle and stand (t minutes).
5. Add 2 mL diethyl ether and agitate for 30 seconds (Vortex mixer).
6. Withdraw 3.0 μL portion of ether and analyze by GC/AFID or GC/FID.
7. A solution of known concentration is used as a standard.
8. The effectiveness of clays having amines attached with and without Cu^{+2} in decomposing DECP is shown in Figure 2.

4-MINUTE MODIFICATION

1. Multiple 200 mg samples of clay were treated with 5, 10, 20, 30, 40, 50 or 60 μL of neat CEES or DECP.
2. The vial was allowed to stand a total of 4.0 min including mixing time.
3. Analysis as above gave results such as those in Figure 3 expressed as unrecovered or irreversibly bound CEES.

VALIDATION OF THE "4-MINUTE" EXPOSURE-EXTRACTION TEST

1. One extraction gave complete removal of simulant

   a. By analysis of second extract

The test was run with Fuller's Earth as above with 60 μL of CEES. The extract recovered from addition of 2 mL of ether was measured as 1.50 mL and contained 26.7 μL CEES/mL or 89.0% of the spike. The remaining 0.50 mL should contain 13.3 μL of CEES which on addition of 2 mL of ether would give a solution of concentration of 5.32 μL of CEES/mL. The GC determination gave 4.0 μL CEES/mL.

   b. By microextraction principal

Utilizing the method of Rhoades and Nulton (*J. Environ. Sci. Health,* A15(5):467-84, 1980), the completeness of a single, 2 mL extraction was studied. The concentration of the first 2 mL was found to be 26.1 μL of CEES/mL, and with addition of 2 mL more of ether, the concentration was exactly 50% or 13.1 μL CEES/mL as determined by GC analysis. Thus, this second method also confirmed that a single, 2 mL ether extraction removed all recoverable CEES.

2. Reproducibility of the "4-minute"test

The test procedure above was run with Fuller's Earth as described above and gave the results below:

	Conc of CEES, μL/mL		
Replicate	10 μL Spike	30 μL Spike	60 μL Spike
1	1.41	11.8	26.7
2	1.20	11.6	26.1
3	1.20	11.9	25.7
4	1.09	12.3	25.6
5	1.09	11.4	25.4
Standard Deviation	±0.13	0.34	0.51

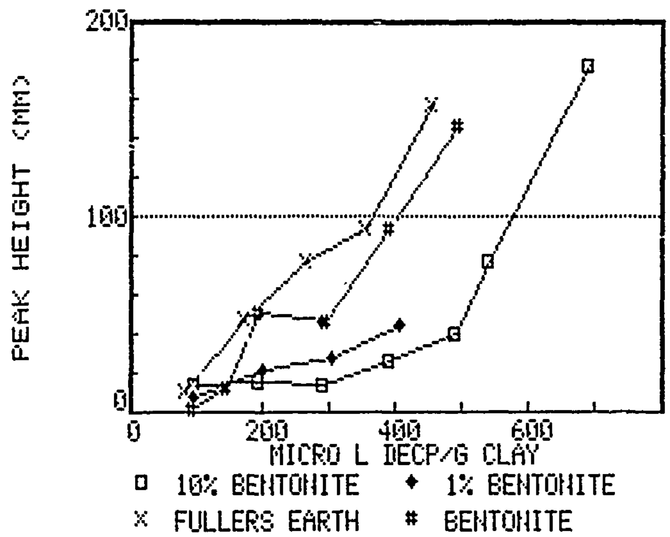


Figure 1. Treated Clays with DECP

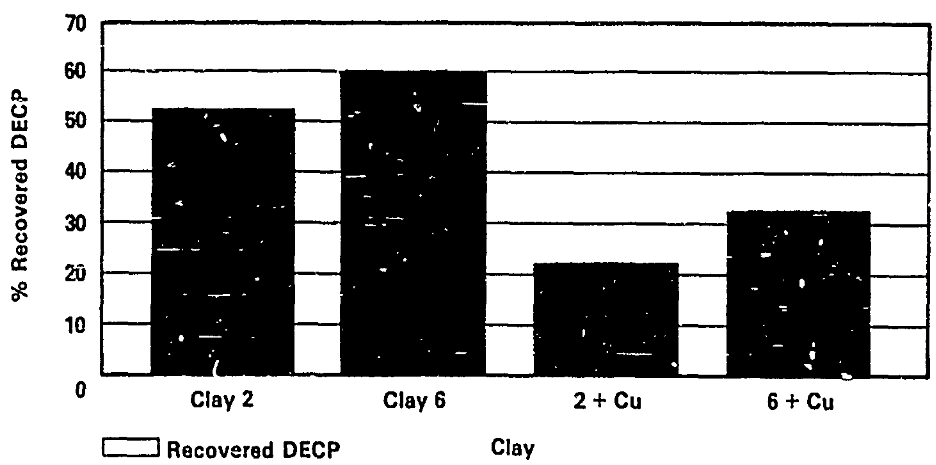


Figure 2. The effect of Copper Ion on the Decomposition of DECP. Clay 2 has $(CH_2)_3$-$NH(CH_2)_2NH_2$ attached to Clay 6, the control clay.

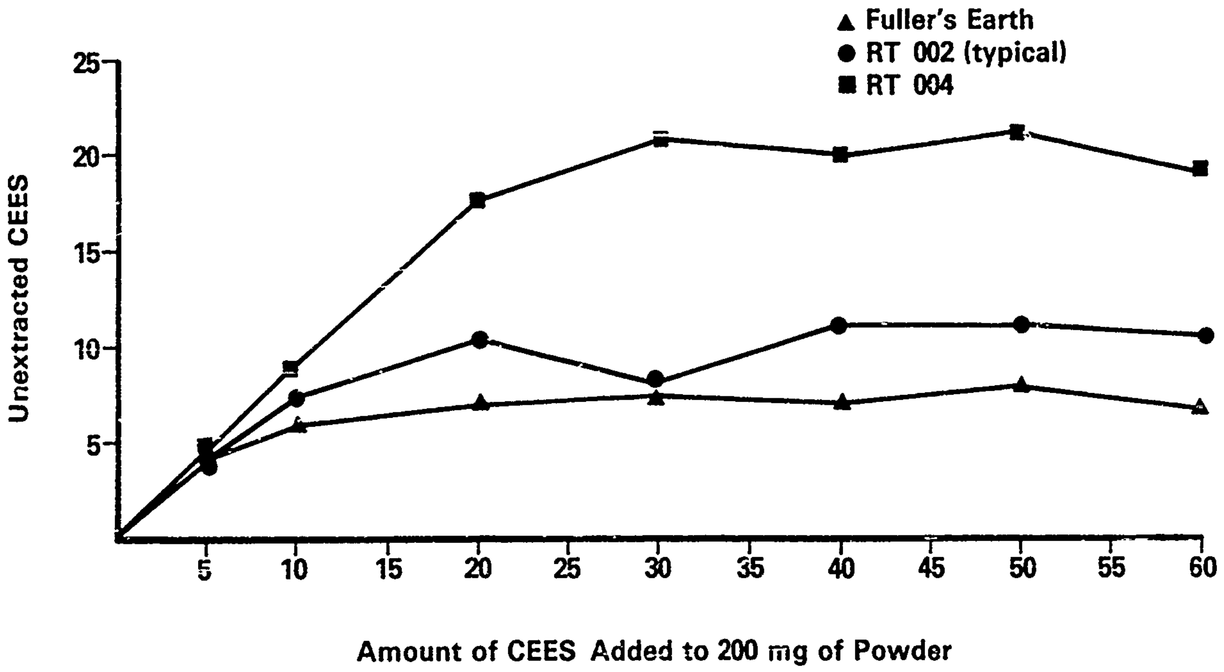


Figure 3. Decomposition of CEES by Clays

TABLE 1. THE RESULTS OF THE WASHER TEST WITH MODIFIED CLAYS

Clay	CEES: Breakthrough Time Average of Three Determinations (min:sec)	CEES: Total Simulant Added (μL)	DECP: Breakthrough Time Average of Three Determinations (min:sec)	DECP: Total Simulant Added (μL)
Acid Wash, 4952	26:36	70	51:50	310
Acidified Surface, 4953	26:50	70	49:40	290
Al cation intercalated, 4954	9:10	30	46:00*	270
Ethylenediamine treated 4955	23:50	60	43:35	250
Beneficiated, calcined, RT004	34:20	90		
Fuller's Earth	13:00	40	30:00	190

*The value for a single run since the replicate runs were invalid due to the failure of the pellets.

SUMMARY OF EVALUATION METHODS

Using any of the several methods for comparing the effectiveness in adsorbing and/or decomposing simulants, it is clear that several of the modified clays are more efficient than Fuller's Earth. The headspace analysis and washer test suggested this relationship; however, the more reliable method of comparing the total adsorption and decomposition of the simulants by the powders seems to be the wicking test described in Poster 102. The results suggest that Anglo-American Clay Corporation (AACC) Sample 004 is significantly more effective than Fuller's Earth in adsorbing blister or nerve agent simulants.

The "4-minute extraction test" has been found to be the more effective method for comparing the decomposition of simulant. Using this method, the AACC Sample 004 was shown to be more effective in irreversibly binding the mustard simulant DEES than was Fuller's Earth.

CONCLUSIONS

1. The modified clays have a very high capacity for adsorption-decomposition of simulants CEES and DECP. The beneficiated or copper-treated clays have significantly greater sorptive ability than that of Fuller's Earth.
2. The clays interact with CEES and DECP irreversibly, probably by a surface reaction, for no reaction product was extractable. Clays modified by beneficiation or treatment with copper gave the greatest reactivity which was several times greater than that of Fuller's Earth.
3. Clays provide a potential decontamination system which is rapid, nontoxic, and binds the agent in an unextractable form.
4. Effective modified clays have been prepared as prototypes; however, additional procedures are evident for providing more highly reactive powders and powders which indicate the presence of simulant or agent.

POSSIBLE MODES OF USE OF CLAYS

1. As the powder to pour on contaminated area
2. In a suspension in a salve or film as agent barrier
3. In a pad or mit for rubbing on contaminated area

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USE OF IN VITRO SKIN PENETRATION SYSTEM TO ASSESS EFFECTS OF SKIN PROTECTION/DECONTAMINATION FORMULATIONS

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ABSTRACT

USING FRESHLY EXCISED PIG OR HUMAN SKIN AND AN IN VITRO SKIN PENETRATION APPARATUS PREVIOUSLY DESCRIBED, THE INFLUENCE OF EACH OF SEVERAL PRETREATMENTS (A PREPARATION OF HOG KIDNEY DFPASE, ETHANOLAMINE, AND A FILM FORMING POLYMER) ON THE DISPOSITION OF RADIOACTIVITY FOLLOWING TOPICAL APPLICATION OF TRITIUM OR C-14 LABELED DIISOPROPYLFLUOROPHOSPHATE (DFP) WAS DETERMINED. EXPERIMENTS WERE CONDUCTED OVER A 24 TO 36 HOUR PERIOD FOLLOWING EXPOSURE TO DFP. RADIOACTIVITY WAS RECOVERED FROM AN UPPER LAYER (APPROXIMATELY 100 um CONTAINING THE EPIDERMIS) OF THE SKIN, THE DERMIS, AND THE TISSUE CULTURE MEDIA BATHING THE VISCERAL SIDE OF THE SKIN (PERCUTANEOUS PENETRATION). AS COMPARED TO SKIN WITH NO PRETREATMENT, THE FILM FORMING POLYMER DID NOT AFFECT THE DISPOSITION OF RADIOACTIVITY. PRETREATMENT OF THE SKIN SURFACE WITH HOG KIDNEY DFPASE OR ETHANOLAMINE RESULTED IN SIMILAR OR INCREASED LEVELS OF RADIOACTIVITY APPEARING IN THE TISSUE CULTURE MEDIUM AS COMPARED TO CONTROLS. HOWEVER, ENZYMATIC AS WELL AS RADIOMETRIC ASSAYS OF ONE OF THE ETHANOLAMINE PRETREATMENT TRIALS REVEALED THAT THE ENZYMATIC:RADIOMETRIC RATIO OF DFP ASSAY EQUIVALENTS OF THE TISSUE CULTURE MEDIUM WAS SIGNIFICANTLY LOWER IN THE CASE OF ETHANOLAMINE PRETREATMENT THAN FOR NO PRETREATMENT. THESE RESULTS DEMONSTRATE THE IMPORTANCE OF CHEMICAL (e.g. ENZYMATIC) AS WELL AS RADIOMETRIC ASSAYS IN THE EVALUATION OF REACTIVE SKIN PROTECTION-DECONTAMINATION FORMULATIONS.

INTRODUCTION

THE EFFICIENT DEVELOPMENT OF SKIN PROTECTIVE FORMULATIONS AGAINST CW AGENTS REQUIRES INITIAL IN VITRO SCREENING AGAINST AGENT ANALOGS PRIOR TO TESTING AGAINST SURETY MATERIALS. USING AN IN VITRO SKIN PENETRATION APPARATUS PREVIOUSLY DEVELOPED IN OUR LABORATORY, RADIOMETRIC AND ENZYMATIC ASSAYS WERE INSTITUTED TO CHARACTERIZE THE PERCUTANEOUS PENETRATION OF CARBON 14 LABELED DIISOPROPYL FLUOROPHOSPHATE (DFP). THE EFFECT OF SEVERAL PRETREATMENTS ON THE DISTRIBUTION OR FATE OF DFP IN THIS TEST SYSTEM WAS DETERMINED.

METHODS

WHOLE SKIN WAS REMOVED FROM THE UPPER BACK OF WEANLING YORKSHIRE PIGS IMMEDIATELY AFTER THE ANIMALS WERE SACRIFICED.

SUBCUTANEOUS FAT AND A PORTION OF THE DERMIS WERE REMOVED WITH A DERMATOME TO GIVE A SKIN THICKNESS OF 1 mm.

SKIN WAS MOUNTED, VISCERAL SIDE DOWN, ON THE PENETRATION CELL AND A FLOW OF TISSUE CULTURE MEDIA THROUGH THE PENETRATION CELL WAS STARTED.

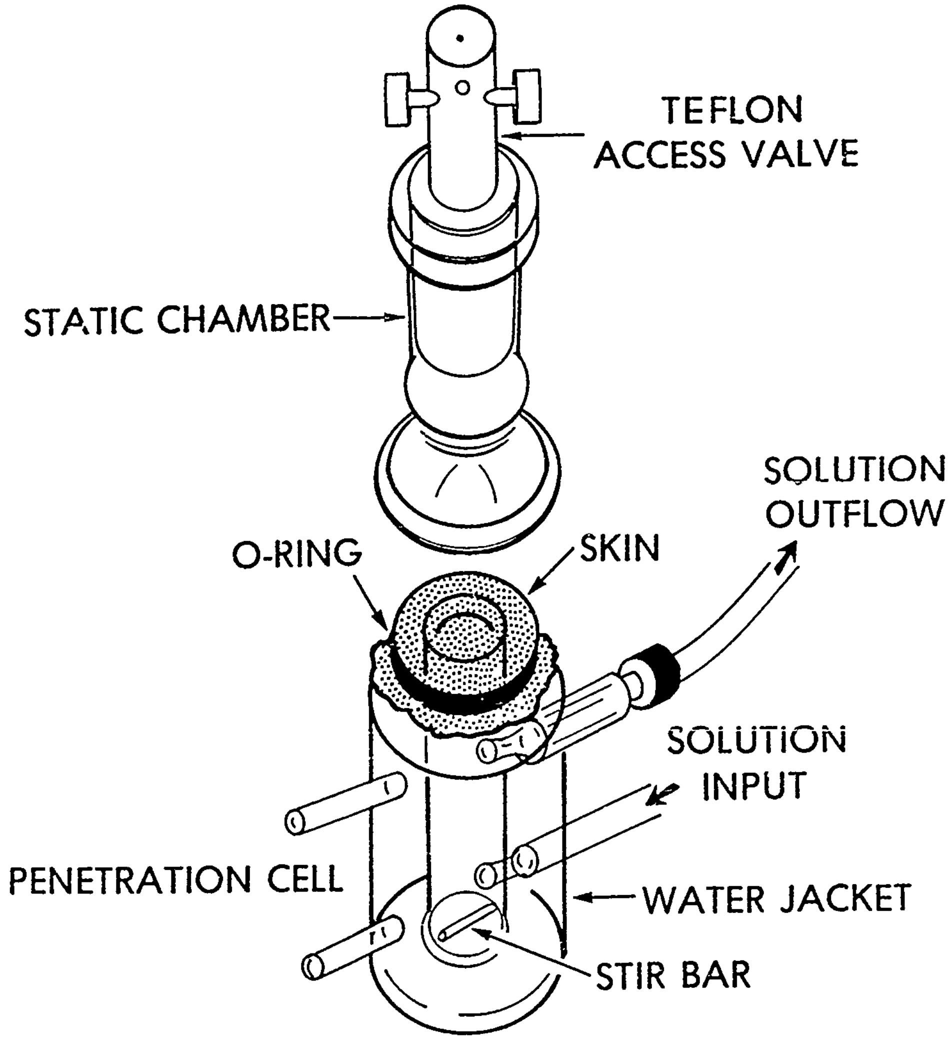
PRETREATMENTS WERE APPLIED TO THE OUTER SKIN SURFACE ON THREE CELLS WHILE THE REMAINING THREE CELLS HAD NO PRETREATMENT (CONTROL).

A STATIC CHAMBER WITH VALVE WAS PLACED OVER THE APPLICATION SIDE OF THE SKIN.

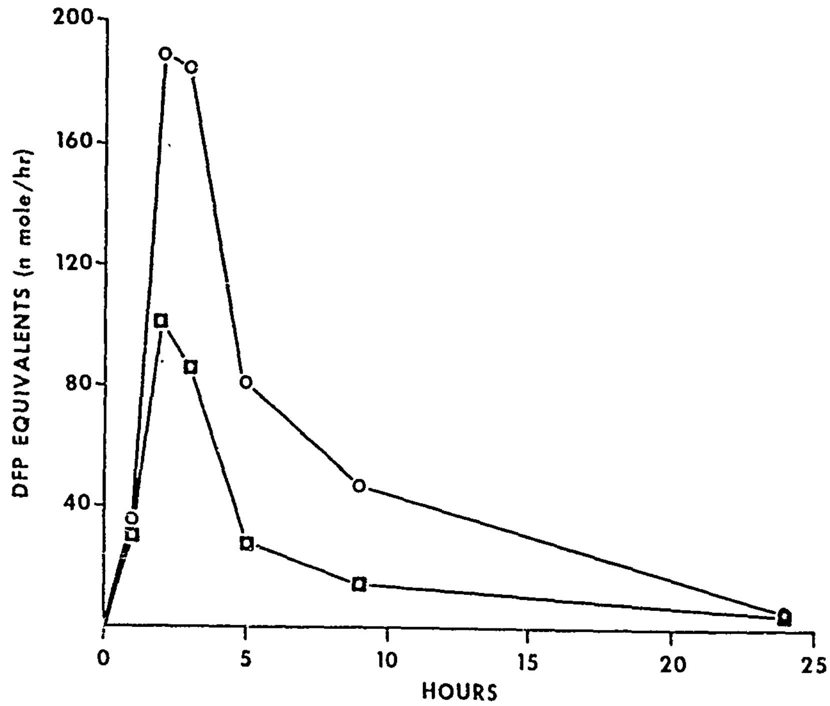
CARBON 14 LABELED DIISOPROPYL FLUOROPHOSPHATE (DFP) WAS APPLIED TO THE SKIN SURFACE WITH A BLUNT TIPPED SYRINGE INSERTED THROUGH THE OPENED VALVE OF THE STATIC CHAMBER.

THE FLOW FROM THE PENETRATION CELL WAS FRACTIONATED INTO HOURLY SAMPLES OVER 24 TO 36 HOURS. AT SELECTED TIME POINTS, ALIQUOTS OF SAMPLES WERE IMMEDIATELY ANALYZED FOR DFP BY ENZYMATIC ASSAY. ALL SAMPLES WERE ASSAYED FOR RADIOACTIVITY.

AT THE END OF THE EXPERIMENT, AN UPPER 100 um LAYER (EPIDERMIS PLUS SOME DERMIS) WAS SEPARATED FROM THE REMAINING DERMIS WITH A FREEZING MICROTOME; THE TWO LAYERS WERE ASSAYED SEPARATELY FOR RADIOACTIVITY USING A SAMPLE OXIDIZER.


TEFLON
ACCESS VALVE
STATIC CHAMBER
SOLUTION
OUTFLOW
O-RING
SKIN
SOLUTION
INPUT
PENETRATION CELL
WATER JACKET
STIR BAR

A-983



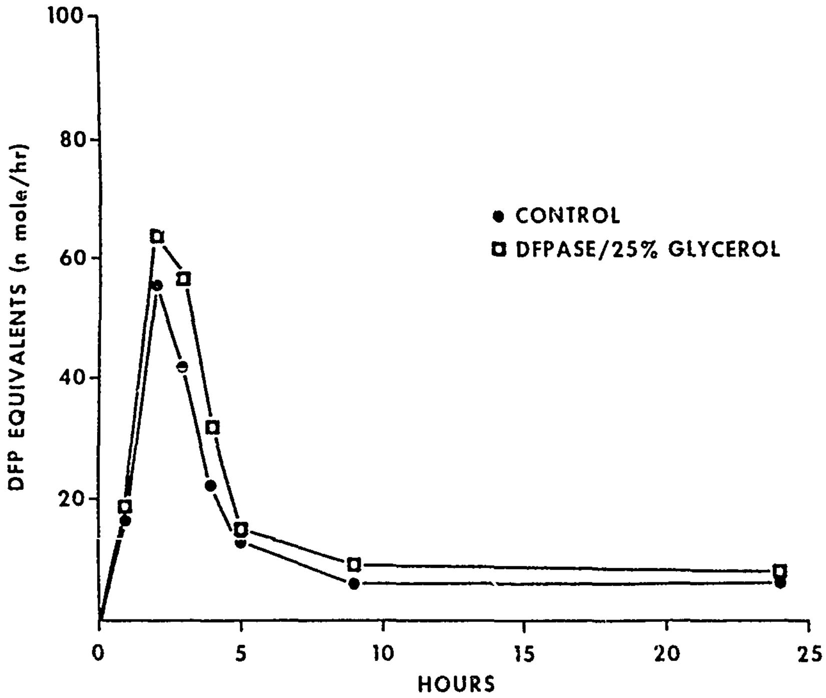
PENETRATION OF RADIOLABELED DFP THROUGH PIG SKIN
AS MEASURED BY RADIOMETRIC (o) AND ENZYMATIC ASSAYS (□)

EFFECT OF FILM FORMING POLYMER PRETREATMENT ON THE

DISPOSITION OF RADIOLABELED DFP IN VITRO

		PERCENT OF APPLIED RADIOACTIVE DOSE OF DFP		
TRIAL[1]	PRETREATMENT	UPPER SKIN LAYER	DERMIS	PERCUTANEOUS PENETRATION
1	None	14±4	2±1	18±4
2	"	12±7	3±1	14±3
1	Polymer	14±1	1±1	15±4
2	"	11±5	3±1	14±2

[1] Trial 1 was conducted with human skin and trial 2 was conducted with pig skin

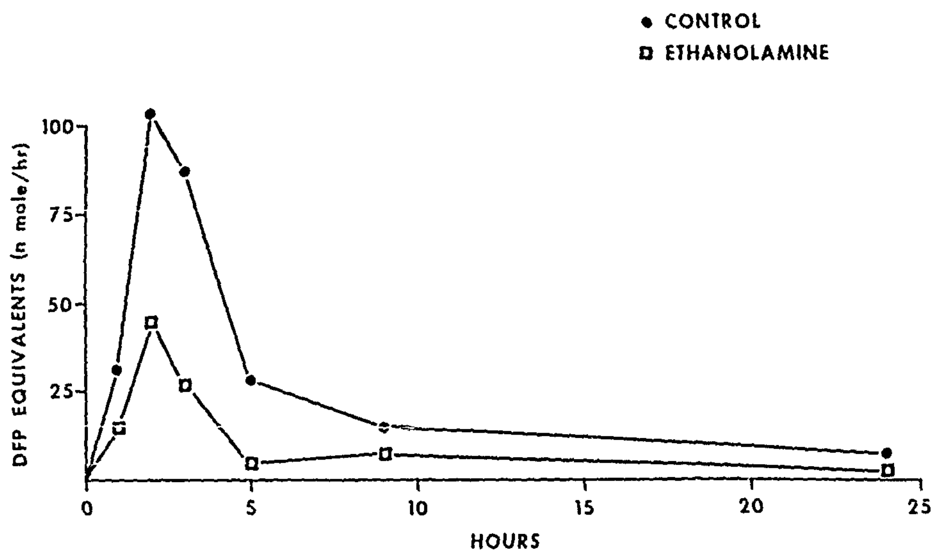


PENETRATION OF ACTIVE DFP THROUGH PIG SKIN PRETREATED WITH DFPASE IN 25% GLYCEROL (◘) VERSUS NO PRETREATMENT (●)

EFFECT OF DFPASE PRETREATMENT ON THE DISPOSITION OF RADIOLABELED DFP IN VITRO

		PERCENT OF APPLIED RADIOACTIVE DOSE OF DFP		
TRIAL[1]	PRETREATMENT	UPPER SKIN LAYER	DERMIS	PERCUTANEOUS PENETRATION
1	None	8±1	1±1	23±2
2	"	3±1	1±1	13±1
3	"	17±2	2±1	9±2
1	DFPASE	8±3	2±1	16±5
2	"	5±1	1±1	15±7
3	"	17±2	3±1	13±1

[1] Trial 1 was conducted on pig skin with neat DFP application (1 mg/cm^2) and 2.4 I.U. DFPASE in Tris buffer. Trial 2 was conducted on pig skin with dilute DFP application (1 mg/cm^2 in ethanol) and 2.4 I.U. DFPASE in 25% glycerol. Trial 3 was conducted on human skin with dilute DFP application (1 mg/cm^2 in ethanol) and 6 I.U. DFPASE in 25% glycerol.



PENETRATION OF ACTIVE DFP THROUGH PIG SKIN PRETREATED WITH ETHANOLAMINE (◻) VERSUS NO PRETREATMENT (●)

EFFECT OF ETHANOLAMINE PRETREATMENT ON THE DISPOSITION OF RADIOLABELED DFP IN VITRO

		PERCENT OF APPLIED RADIOACTIVE DOSE OF DFP		
TRIAL	PRETREATMENT	UPPER SKIN LAYER	DERMIS	PERCUTANEOUS PENETRATION
1	None	13±2	4±1	22±3
2	"	3±1	2±1	38±2
3	"	3±1	2±1	29±3
1	Ethanolamine	18±1	2±1	39±4
2	"	10±2	2±1	48±5
3	"	25±6	4±3	31±7

RESULTS

WHEN RADIOLABELED DFP ALONE WAS APPLIED TO PIG SKIN, APPROXIMATELY 50% OF THE LABEL THAT PENETRATED THE SKIN WAS FOUND TO BE INACTIVATED, AS INDICATED BY ENZYMATIC ASSAY.

SKIN PRETREATMENT WITH HOG KIDNEY DFPASE IN SIMPLE FORMULATIONS (AQUEOUS GLYCEROL OR TRIS BUFFER SOLUTION) WAS NOT EFFECTIVE IN PROVIDING PROTECTION AGAINST DFP PENETRATION, AS MEASURED BY RADIOMETRIC AND ENZYMATIC ASSAYS.

A FILM FORMING POLYMER, USEFUL IN PREVENTING ABSORPTION OF MOSQUITO REPELLENTS, HAD NO EFFECT ON THE DISTRIBUTION OF RADIOACTIVITY FOLLOWING TOPICAL APPLICATION OF RADIOLABELED DFP.

ETHANOLAMINE PRETREATMENT OF THE SKIN INCREASED THE INACTIVATION OF DFP FROM APPROXIMATELY 50% TO 80% DURING THE PEAK TIME INTERVAL (HOURS 1-10) OF PERCUTANEOUS PENETRATION, AS INDICATED BY THE DIFFERENCE IN RADIOMETRIC AND ENZYMATIC ASSAYS.

CONCLUSIONS

ENZYMATIC ASSAY, IN COMBINATION WITH RADIOMETRIC MEASUREMENTS OF PERCUTANEOUS ABSORPTION, WERE USEFUL IN THE CHARACTERIZATION OF REACTIVE SKIN PROTECTION FORMULATIONS.

CATALYSTS FOR THE HYDROLYSIS OF ORGANOPHOSPHORUS COMPOUNDS, SUCH AS DFPASE, MAY REQUIRE SPECIAL FORMULATION TO EXERT A PROTECTIVE EFFECT WHEN PLACED ON THE SKIN.

PRELIMINARY RESULTS INDICATE THAT ETHANOLAMINE MAY BE A USEFUL COMPONENT OF TOPICAL FORMULATIONS FOR PROPHYLAXIS AGAINST CW AGENTS.

FURTHER COMPARISON OF IN VIVO AND VITRO SKIN PENETRATION

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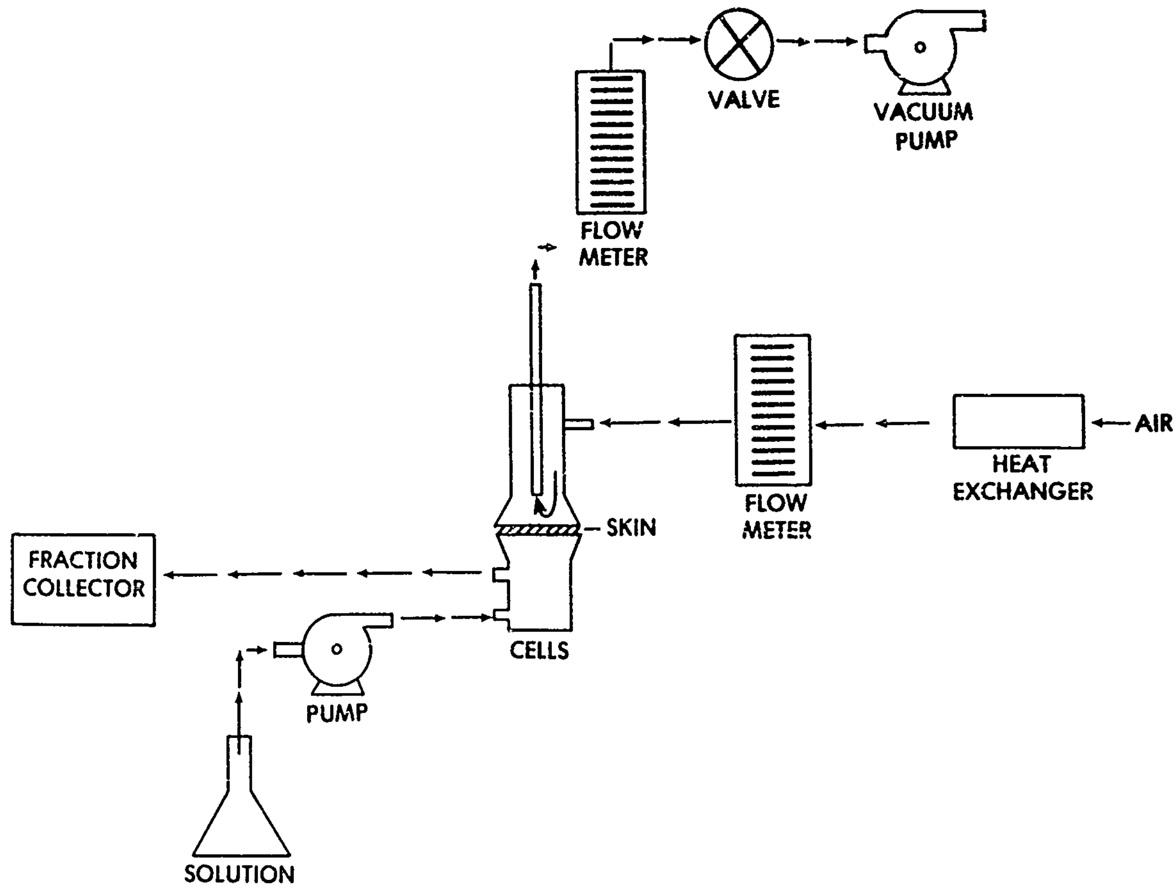
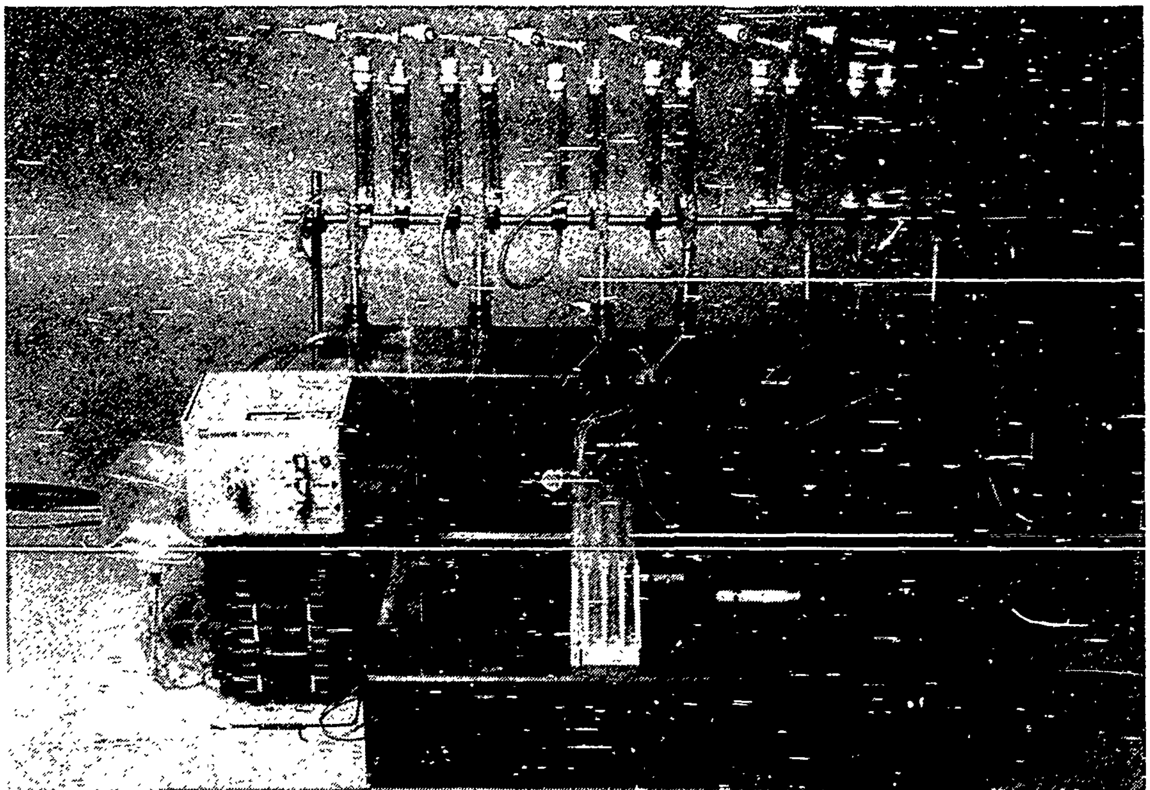
Letterman Army Institute of Research, Presidio of San Francisco, CA 94129

ABSTRACT

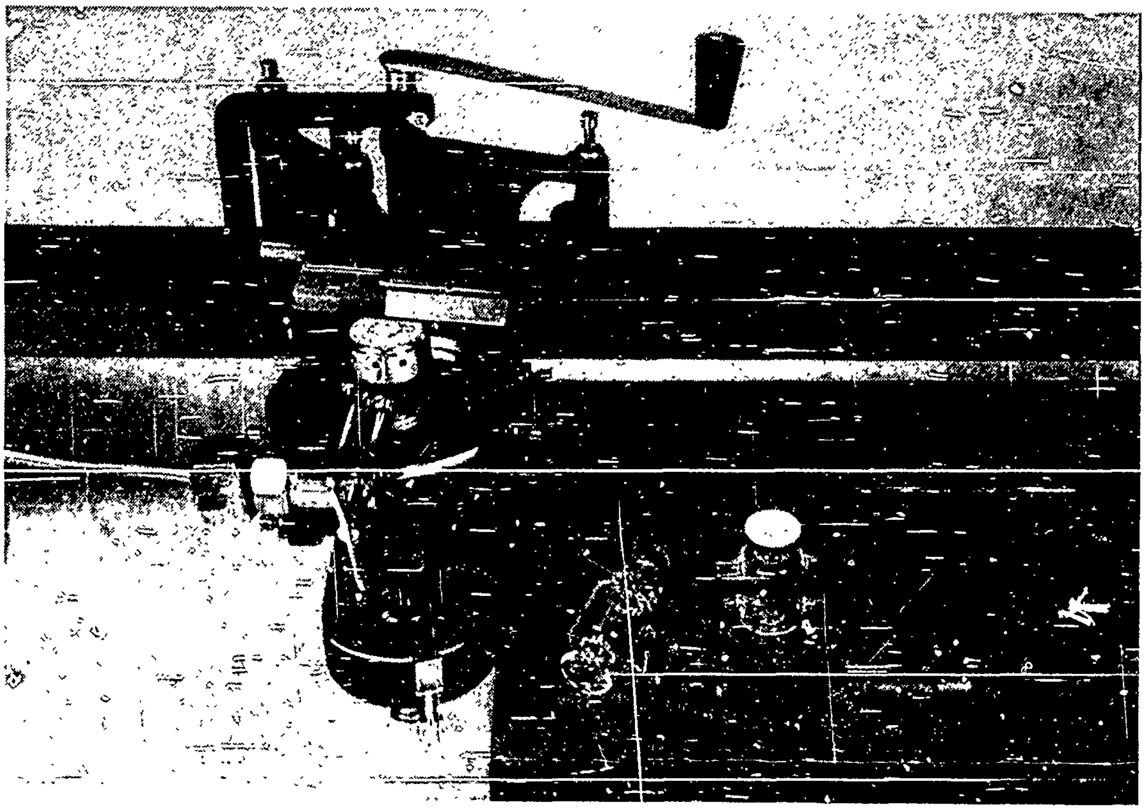
USING A PREVIOUSLY DESCRIBED SYSTEM, TECHNIQUES FOR THE DETERMINATION OF SKIN PENETRATION AND EVAPORATION IN VITRO WERE IMPROVED TO INCLUDE SEPARATE DETERMINATIONS FOR COMPOUND RESIDUES IN THE EPIDERMIS, DERMIS AND OUTER SKIN WHICH DOES NOT CONTACT THE FLUID INSIDE THE PENETRATION CELL. A MICROTOME WITH THE CAPACITY TO FREEZE SKIN SAMPLES WAS USED TO SEPARATE THE EPIDERMIS FROM THE DERMIS AND A CORK BORER WAS USED TO REMOVE THE OUTER SKIN. THE ADDITION OF DERMAL VALUES TO SPLIT THICKNESS VALUES PROVIDED AN ADJUSTED VALUE. COMPARISONS BETWEEN THE PERCENT PENETRATION VALUES FOR NINE COMPOUNDS WERE MADE BETWEEN PIG SKIN IN VIVO AND IN VITRO FOR FULL THICKNESS, SPLIT THICKNESS AND ADJUSTED SPLIT THICKNESS VALUES. THE SPLIT THICKNESS VALUE PROVIDED A BETTER ESTIMATE OF IN VIVO PENETRATION THAN THE FULL THICKNESS VALUE; HOWEVER, THE ADJUSTED VALUE PROVIDED THE BEST OVERALL ESTIMATE OF PENETRATION FOR THE NINE COMPOUNDS. THE PENETRATION OF FIVE COMPOUNDS THROUGH FRESH PIG SKIN AND PIG SKIN WHICH WAS DELIBERATELY FROZEN AND EXPOSED TO ETHYLENE OXIDE VAPOR WAS DETERMINED IN AN EFFORT TO DETECT AN INFLUENCE OF SKIN VIABILITY ON PENETRATION VALUES. NO SIGNIFICANT DIFFERENCES WERE OBSERVED. FINALLY, THE INFLUENCE OF AIR FLOW ON THE IN VITRO PENETRATION AND EVAPORATION OF SIX COMPOUNDS ON PIG SKIN WAS DETERMINED BY COMPARING THESE VALUES AT AIR FLOWS OF 60mL/MIN. AND 600mL/MIN. AS EXPECTED, MUCH GREATER EVAPORATION OCCURRED AT THE GREATER AIR FLOW WITH A SIMULTANEOUS REDUCTION IN PENETRATION. A HIGHER AIR FLOW RESULTS IN LESS PENETRATION WHICH MORE CLOSELY APPROXIMATES PENETRATION IN VIVO.



IN VITRO SKIN PENETRATION-EVAPORATION SYSTEM
(SPE SYSTEM)



IN VITRO SKIN PENETRATION - EVAPORATION SYSTEM



EFFECT OF IN VITRO METHOD ON TWO MEASURES OF AGREEMENT BETWEEN IN VITRO AND IN VIVO PIG SKIN PENETRATION VALUES FOR NINE COMPOUNDS.

	MEASURE OF AGREEMENT	
IN VITRO METHOD	CORRELATION COEFFICIENT	$\left| \sum_{i=1}^{9} PR - 1 \right|^{a}$
WHOLE SKIN	0.58	47
SPLIT SKIN	0.44	11
ADJUSTED SPLIT SKIN	0.51	6
600ml/min AIR FLOW ADJUSTED SPLIT SKIN	0.79 [b]	5

[a] SUM OF THE ABSOLUTE VALUES OF EACH $\frac{\text{in vivo}}{\text{in vitro}}$ PENETRATION RATIO MINUS ONE.

[b] SIGNIFICANT p= 0.05 .

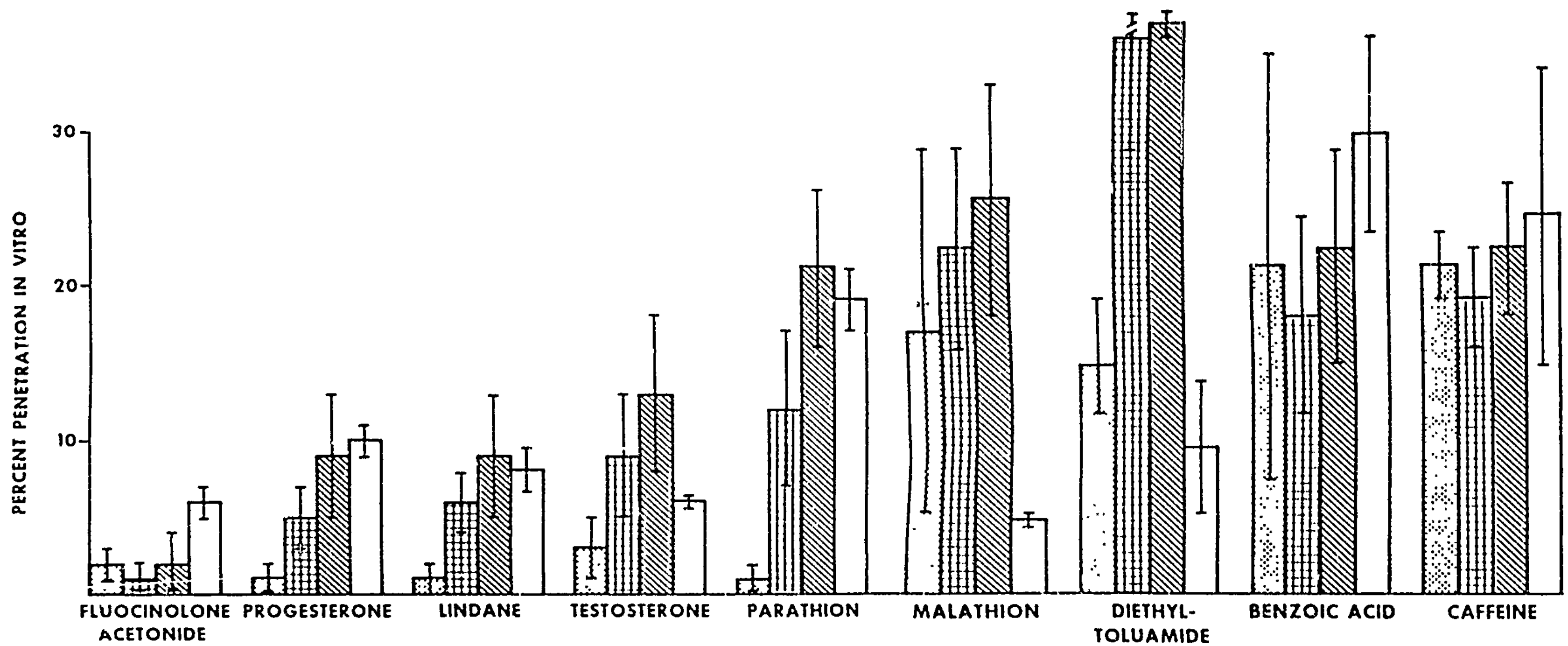
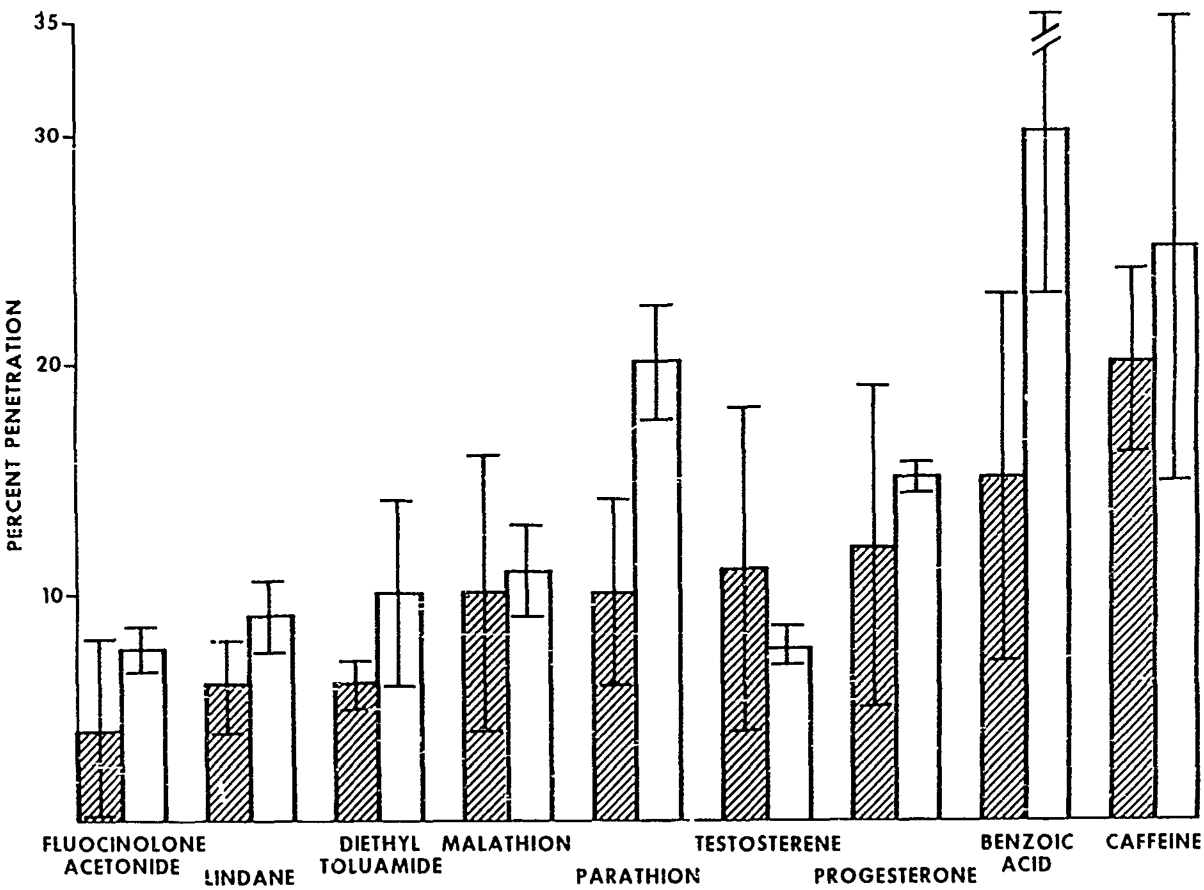


Fig. 2. PIG SKIN PENETRATION VALUES OBTAINED FROM FULL THICKNESS, SPLIT THICKNESS AND SPLIT THICKNESS PLUS DERMAL RECOVERY, VERSUS IN VIVO.



ADJUSTED PIGSKIN PENETRATION VALUES OBTAINED IN VITRO (▨) WITH INCREASED AIRFLOW VS IN VIVO (□)

TABLE 3 THE EFFECT OF ETHYLENE OXIDE AND FREEZING ON SKIN PENETRATION VALUES*

COMPOUND	PERCUTANEOUS PENETRATION	EVAPORATION	EPIDERMIS	DERMIS
BENZOIC ACID	$\frac{17\pm6}{19\pm13}$	$\frac{25\pm15}{17\pm14}$	$\frac{34\pm9}{44\pm7}$	$\frac{4\pm4}{2\pm2}$
CAFFEINE	$\frac{18\pm3}{14\pm10}$	$\frac{4\pm2}{4\pm1}$	$\frac{35\pm20}{60\pm11}$	$\frac{3\pm1}{2\pm1}$
M-DEET	$\frac{26\pm3}{31\pm10}$	$\frac{56\pm5}{52\pm5}$	$\frac{6\pm2}{6\pm3}$	$\frac{1\pm1}{1\pm1}$
LINDANE	$\frac{6\pm2}{5\pm3}$	$\frac{62\pm14}{68\pm7}$	$\frac{14\pm4}{14\pm2}$	$\frac{4\pm2}{5\pm2}$
FLUOCINOLONE	$\frac{2\pm1}{2\pm1}$	N/A	$\frac{75\pm8}{79\pm1}$	$\frac{2\pm1}{4\pm4}$

*MEAN ±1 S.D. OF APPLIED RADIOACTIVE DOSE $\frac{\text{CONTROL}}{\text{TREATED}}$

IN VITRO TESTS TO COMPARE SKIN DECONTAMINANT POWDERS

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RATIONALE

PREFACE

PAST EXPERIMENTS SUGGEST THAT DECONTAMINANT EFFICACY FALLS EXPONENTIALLY AS AGENT CONTACT TIME INCREASES. THEREFORE, DECONTAMINANT POWDERS MUST ABSORB LIQUID AGENTS VERY RAPIDLY TO BE EFFECTIVE. AGENT MUST BE REMOVED FROM HAIR FOLLICLE OPENINGS AND SKIN CREASES BY DETOXIFICATION, DISPLACEMENT OR DILUTION. THE USE OF LIVE ANIMALS TO ASSESS POWDERS BY COMPARING REDUCTIONS OF LETHAL OR IRRITANT EFFECTS IS SLOW, EXPENSIVE, AND INDIRECT. MORE DIRECT MEASUREMENTS OF POWDER UPTAKE RATES, RETENTION CAPACITIES AND AGENT DETOXIFYING PROPERTIES ARE NEEDED. THIS POSTER DESCRIBES ONE APPROACH TO THIS REQUIREMENT.

PROBLEM

MEASURE PARAMETERS FOR SELECTION OF BEST POWDER

APPROACH

IDENTIFY KEY FEATURES FOR SKIN DECONTAMINATION

A. SCENARIO: 1. AGENT DROP ON SKIN

2. REMOVE DROP ASAP

3. USE EXCESS POWDER

B. NEEDS: 1. FAST UPTAKE RATE

2. HIGH CAPACITY (SORB/BIND)

C. POWDERS: 1. HIGH CAPILLARITY

2. LARGE INTERNAL VOLUME

3. HIGH INTERNAL ACTIVITY

DESIGN

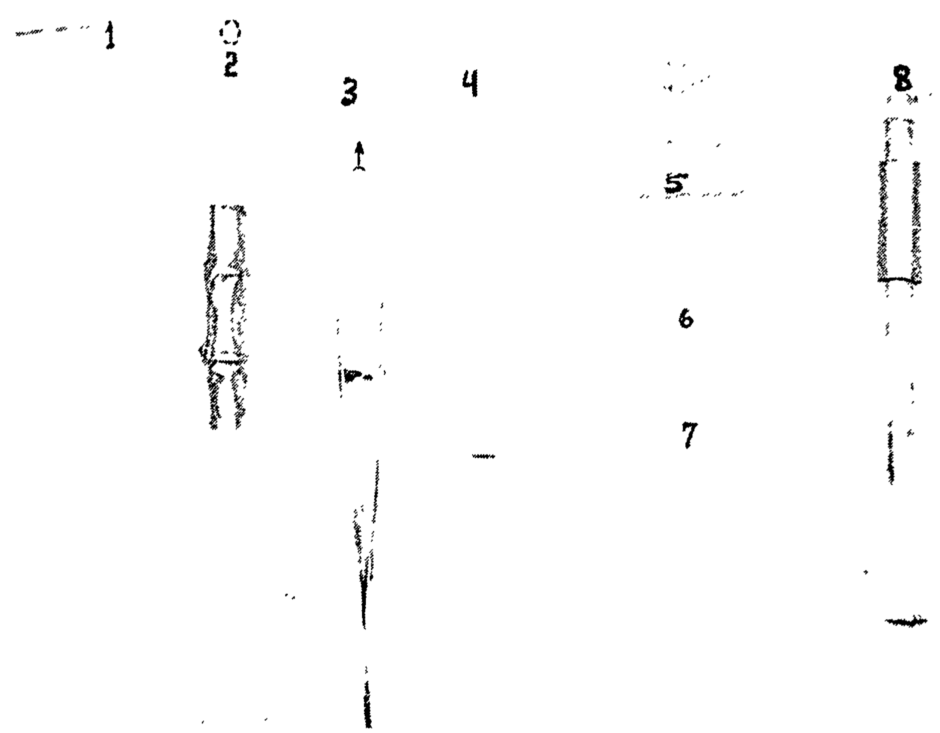
PLAN TEST AND MAKE EQUIPMENT TO DIFFERENTIATE

A. SCENARIO:
1. SMALL HOLE MODELS AGENT DROP
2. TEST FOR 1 MINUTE
3. TUBE HOLDS EXCESS POWDER

B. NEEDS:
1. UPTAKE MEASURED AS AGENT WEIGHT LOSS IN 1 MINUTE
2. CAPACITY AS WEIGHT LOSS AT 1 MINUTE AND END POINT.
3. GLASS TUBE MODELS SKIN

C. POWDERS:
1. MEASURE OR CONTROL SIZES/DISTRIBUTIONS OF PARTICLES
2. FIX POWDER VOLUME, WEIGH AGENT INTAKE AS UPTAKE
3. WATCH FOR ALTERED M-8 COLOR: CLUE OF AGENT PRODUCTS; MAY EXTRACT LOAD FOR ASSAY.

METHOD



KEY TO MICROTUBE PREPARATION

1. CLIP CAP OFF 0.5 ML MICROCENTRIFUGE TUBE.

2. BORE HOLES IN RIM AND TIP OF MICROTUBE. (BORER IS GUARDED 18 GAUGE NEEDLE)

3. PAPER DISC (4 MM) GOES IN MICROTUBE TIP.

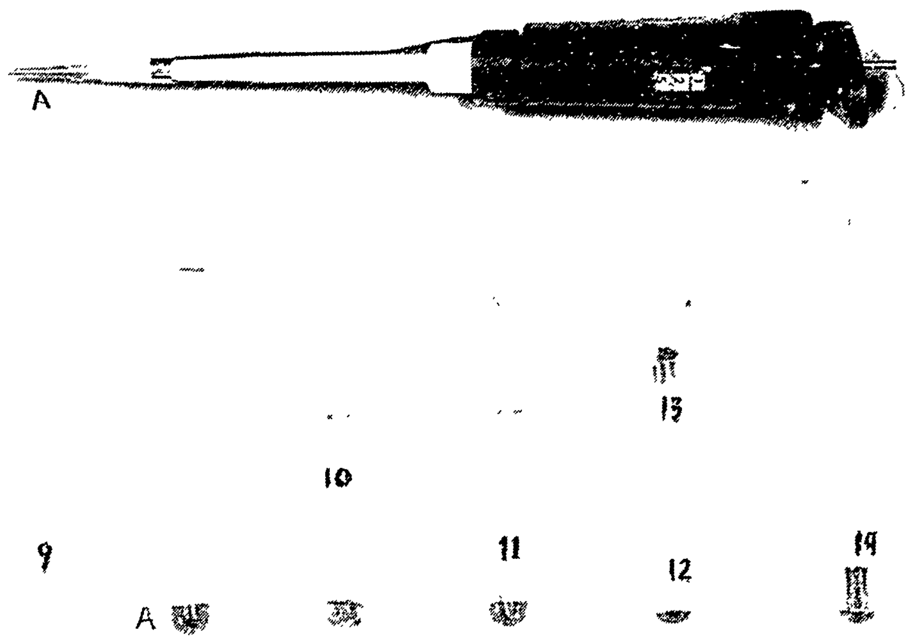
4. TISSUE PAPER DISC PRESSED OVER HOLE.

5. WEIGHED POWDER LOAD, 0.25 ML VOLUME.

6. M-8 PAPER DISC TOPS LOAD SETTLED IN MICROTUBE.

7. MICROTITER PLATE SHAKER SETTLES LOAD IN 5 MIN.

8. LOAD IS PACKED 15 MM DEEP WITH ROD.



KEY TO MICROTUBE POWDER TEST

9. TINNED COPPER WIRE IS HOOKED UNDER RIM.

A. AGENT (SHOWN BLUE, 0.125 ML), IN 12 X 75 MM GLASS TUBE AWAITS GROSS WEIGHT DETERMINATION.

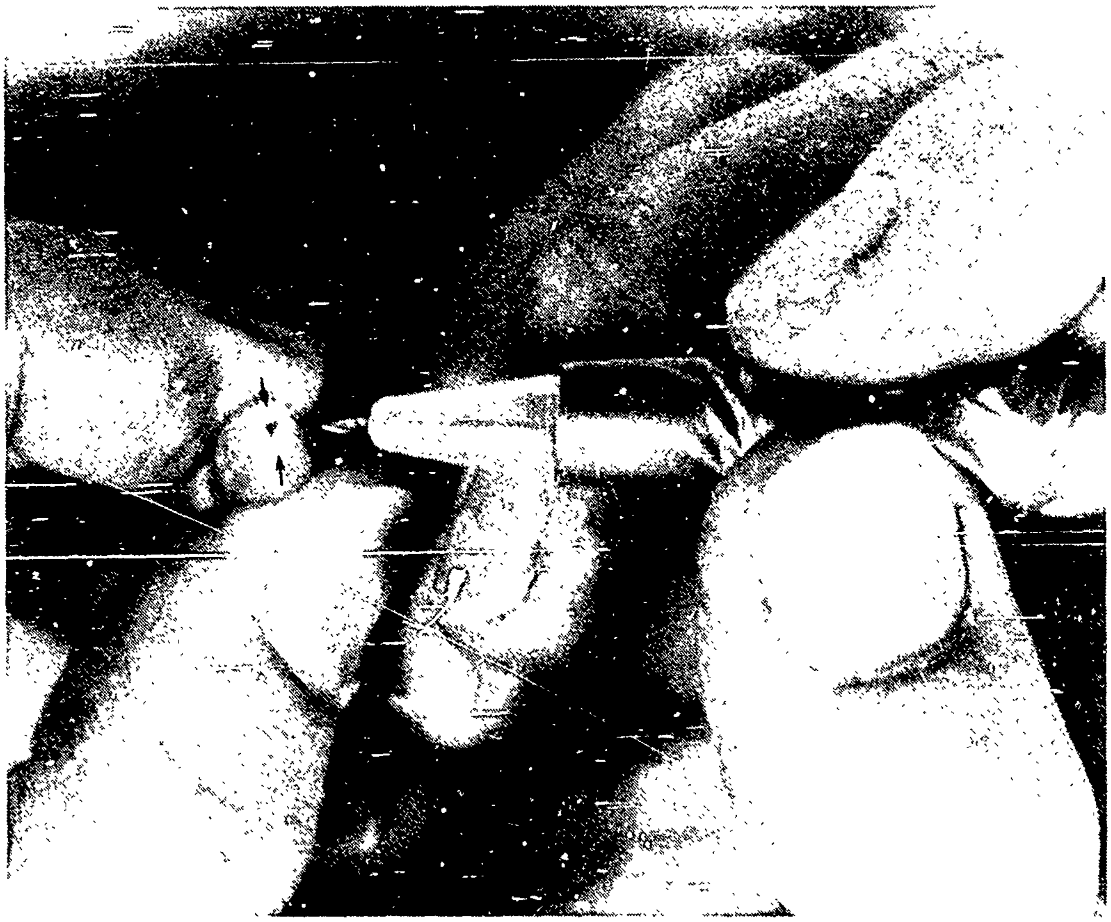
10. LOADED MICROTUBE IS SECURED ABOVE AGENT.

11. UNBENT WIRE FORCES TIP INTO AGENT FOR 1 MIN. THEN LIFTS TIP TO DRAIN >2 MIN.

12. AGENT RESIDUE AND GLASS AWAIT RE-WEIGHING.

13. MICROTUBE IS WIPED, STORED IN OTHER GLASS DURING RE-WEIGHING, THEN IS RETURNED AND RESUBMERGED.

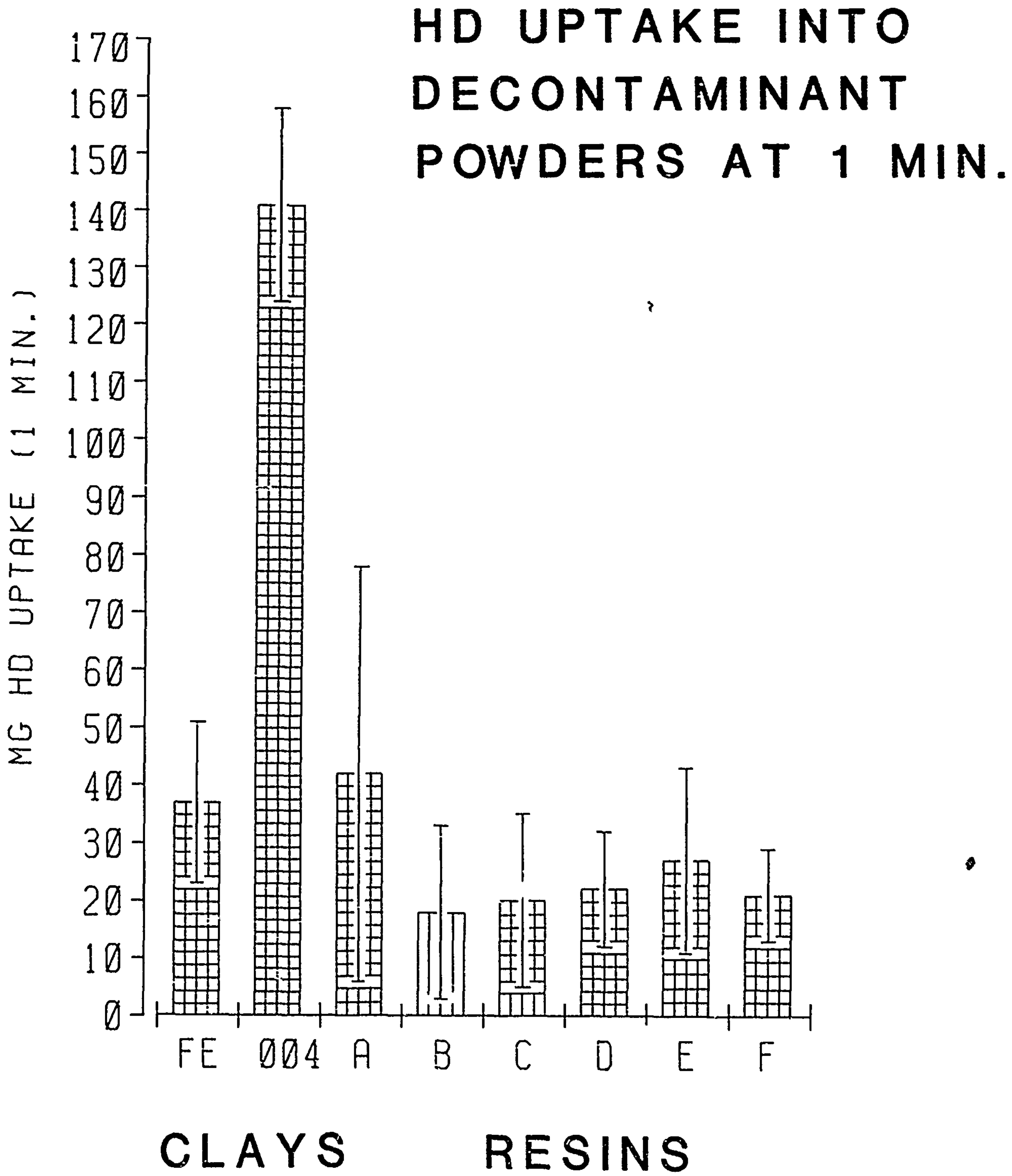
14. RESIDUE UPTAKE IS TIMED FOR COLOR CHANGE IN M-8 PAPER AND/OR COMPLETE UPTAKE.



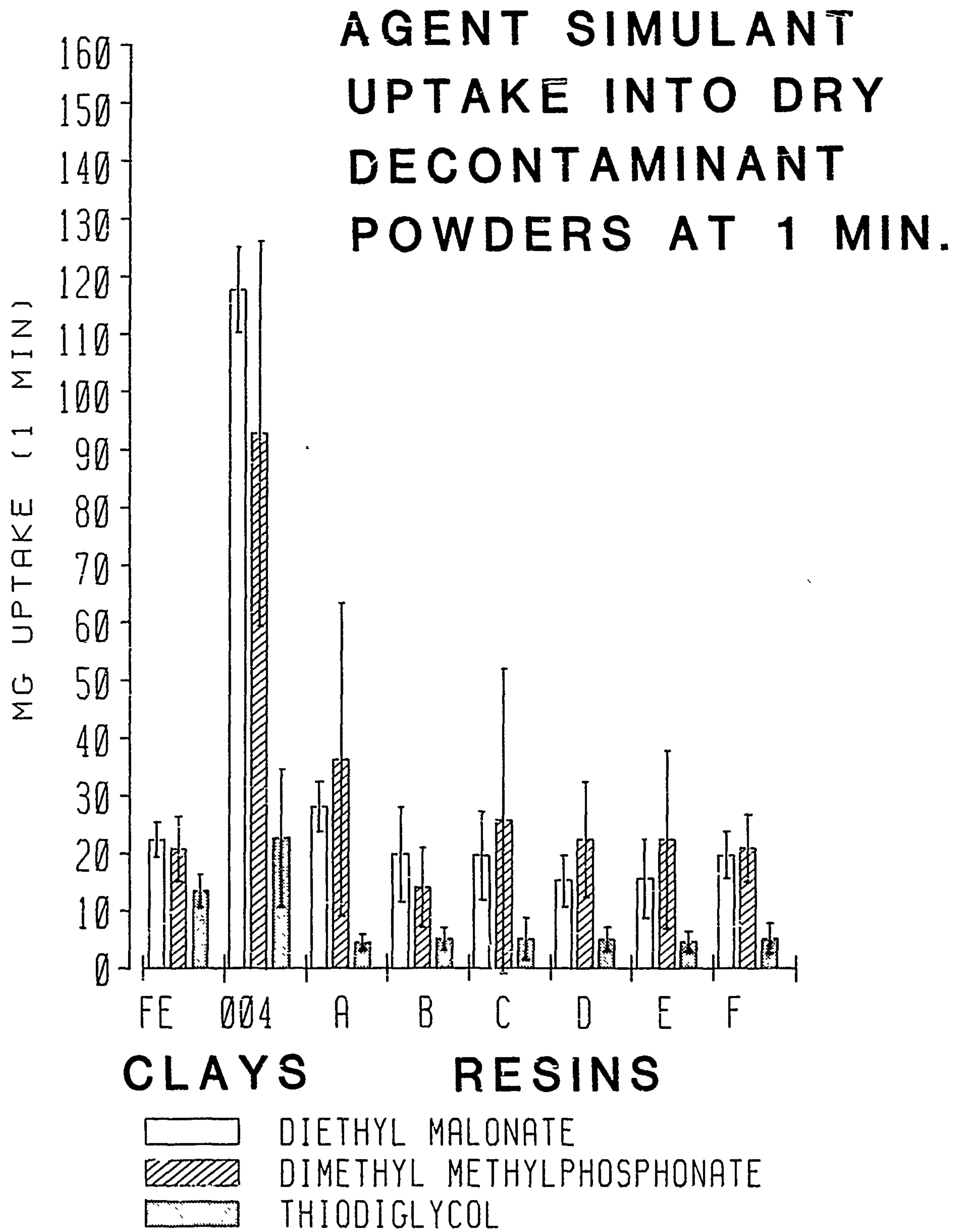
THE DIAMETER OF THE HOLE IN THE MICROTUBE TIP SIMULATES AN AGENT DROP OF ABOUT 1 MM DIAMETER ON SKIN. THIS HOLE IS LARGE ENOUGH NOT TO BE THE LIMITING FACTOR IN AGENT UPTAKE BUT IT IS TOO LARGE TO PREVENT LOSSES OF POWDER. THEREFORE, A SMALL DISC OF TISSUE PAPER IS PRESSED OVER THE HOLE, INSIDE THE MICROTUBE, TO RETAIN ALL POWDER WHILE PASSING AGENT FREELY. ANOTHER MODIFICATION IS MADE TO AVOID SEALING OF THE HOLE AGAINST THE GLASS TUBE; SUCH SEALING IS PREVENTED BY MAKING A NOTCH (OR CUT) THROUGH THE HOLE WITH A SCALPEL BLADE. THIS NOTCH ACCOUNTS FOR THE REFLECTION (SEE ARROWS) AND TRIANGULAR SHAPE OF POLYPROPYLENE ADJACENT TO THE HOLE, AS PHOTOGRAPHED.

VALIDATION

AGENT



THE BAR GRAPH AT LEFT SHOWS THAT THE MICROTUBE TEST METHOD DISCRIMINATES BETWEEN DRY POWDERS ON THE BASIS OF DIFFERENCES IN LIQUID UPTAKE. THE DATA INDICATE THAT CLAY 004 ABSORBS MORE THAN 3 1/2 TIMES AS MUCH SULFUR MUSTARD AGENT (HD) AS IS TAKEN INTO FULLER'S EARTH IN ONE MINUTE. CLAY 004 IS A PURIFIED FULLER'S EARTH THAT HAS BEEN LARGELY STRIPPED OF EXCHANGEABLE IONS AND WATER OF HYDRATION. SIMILAR PATTERNS, SHOWING SIGNIFICANTLY GREATER UPTAKE IN CLAY 004 THAN IN THE OTHER MATERIALS, HAVE BEEN SEEN WITH THE ORGANOPHOSPHATE AGENT VX AND THE ARSENICAL AGENT LEWISITE. THESE RESULTS SUGGEST THAT CLAY 004, LIKE FULLER'S EARTH, IS NON-SPECIFIC IN AFFINITY FOR MOBILE LIQUIDS.


AGENT SIMULANT
UPTAKE INTO DRY
DECONTAMINANT
POWDERS AT 1 MIN.
MG UPTAKE (1 MIN)
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
FE
004
A
B
C
D
E
F
CLAYS
RESINS
DIETHYL MALONATE
DIMETHYL METHYLPHOSPHONATE
THIODIGLYCOL

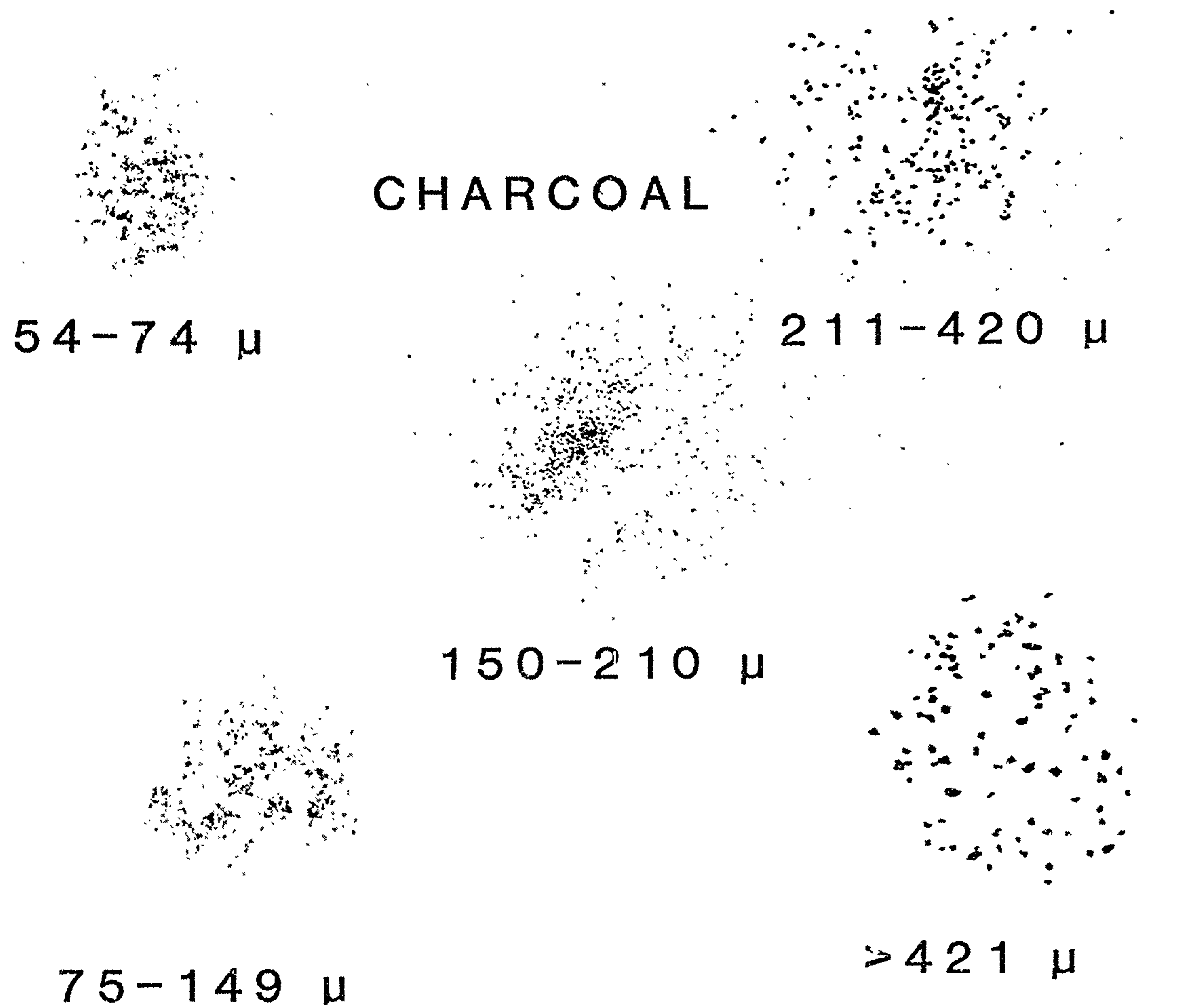
MICROTUBE TEST COMPARISONS OF UPTAKE DATA FOR THREE AGENT SIMULANTS SUGGEST THE FEASIBILITY OF SCREENING CANDIDATE SORBENT DECONTAMINANTS WITH NON-TOXIC MATERIALS. RESULTS, AT LEFT, SHOW DISCRIMINATION PATTERNS SIMILAR TO THOSE OBSERVED WITH TOXIC AGENTS, ALTHOUGH UPTAKE WEIGHTS DIFFER. UPTAKE WEIGHTS APPEAR TO CORRELATE WITH AGENT OR SIMULANT VISCOSITY, RATHER THAN RELATING TO CHEMICAL DIFFERENCES. THIODIGLYCOL, A VISCOUS HYDROLYSIS PRODUCT OF HD, EXHIBITS EXCEPTIONALLY LOW UPTAKE WEIGHTS AND LITTLE DISCRIMINATION AMONG CLAYS AND RESINS.

SIMULANT MICROTUBE TEST

EXPLANATION

EXTERNAL SPACE OF PARTICLES

THE MICROTUBE TEST OFFERS A METHOD TO MEASURE POSSIBLE EFFECTS OF PARTICLE SIZES ON DECONTAMINATION. IT PROVIDES MEANS TO DISTINGUISH BETWEEN LIQUID UPTAKE RATES (WT. SORBED/TIME) AND WICKING RATES (MM/TIME). THESE PARAMETERS CAN BE RELATED TO PARTICLES OF LIKE COMPOSITION BUT DIFFERENT SIZE RANGES OR SIZE DISTRIBUTIONS. INITIALLY, G-SERIES SEPHADEX™ PARTICLES OF UNIFORM COMPOSITION WERE OBSERVED TO DIFFER IN UPTAKES WITH SIZE RANGE. THE PHOTOGRAPH BELOW SHOWS ACTUAL CHARCOAL SIZES/RANGES USED TO OBTAIN THE DATA GRAPHED BELOW. THIS INDICATES CLOGGING WITH FINES, WHICH PASS THROUGH THE FINEST SCREEN. WICKING IS MOST RAPID IN COARSE MATERIAL.



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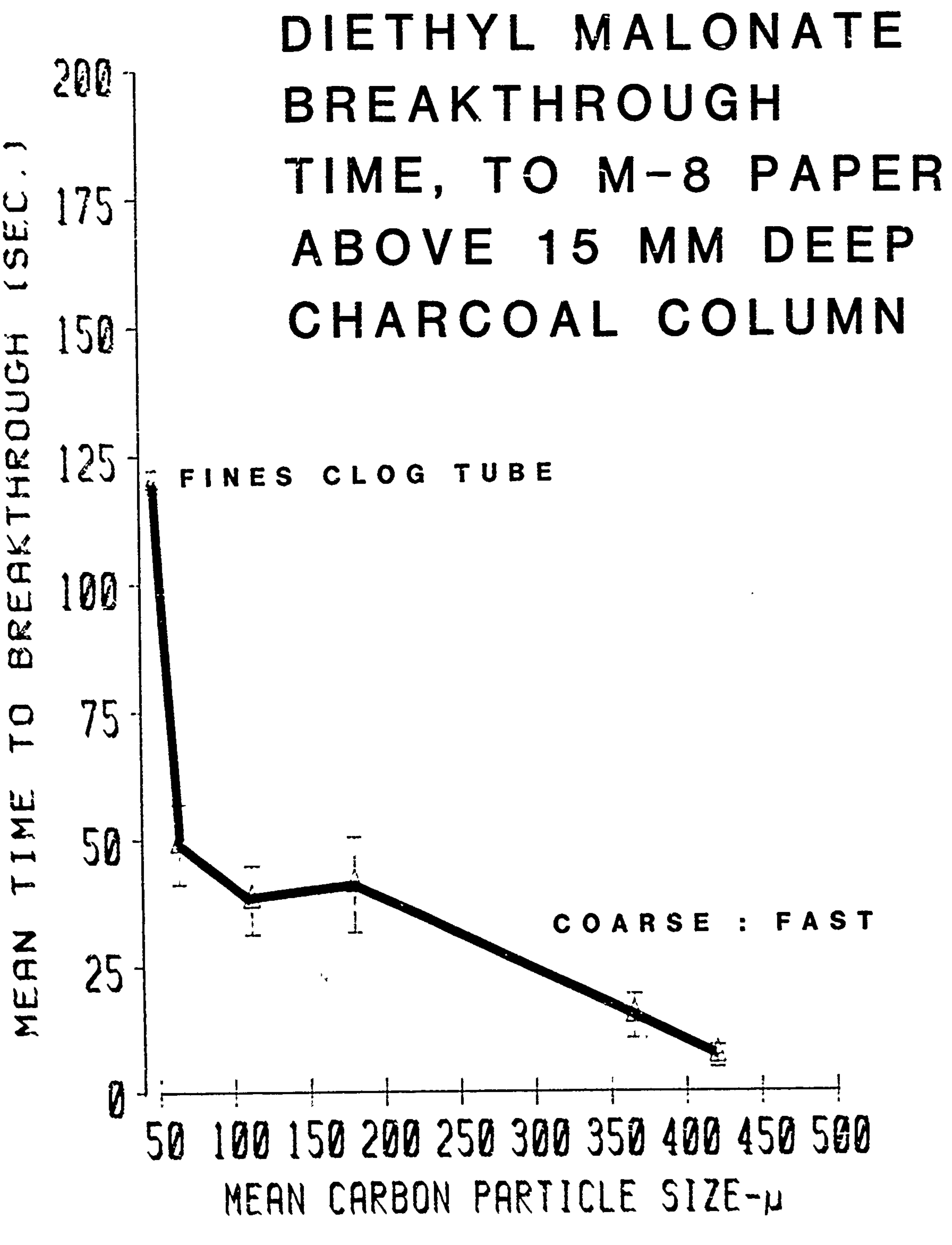
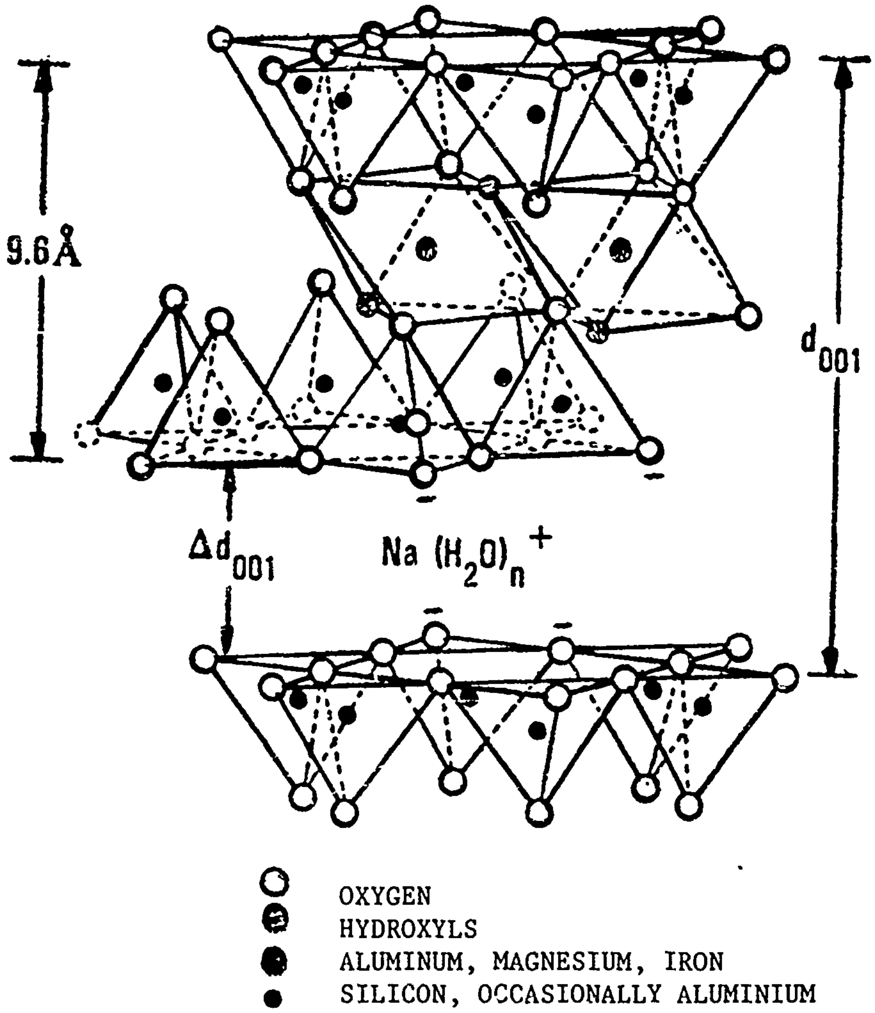
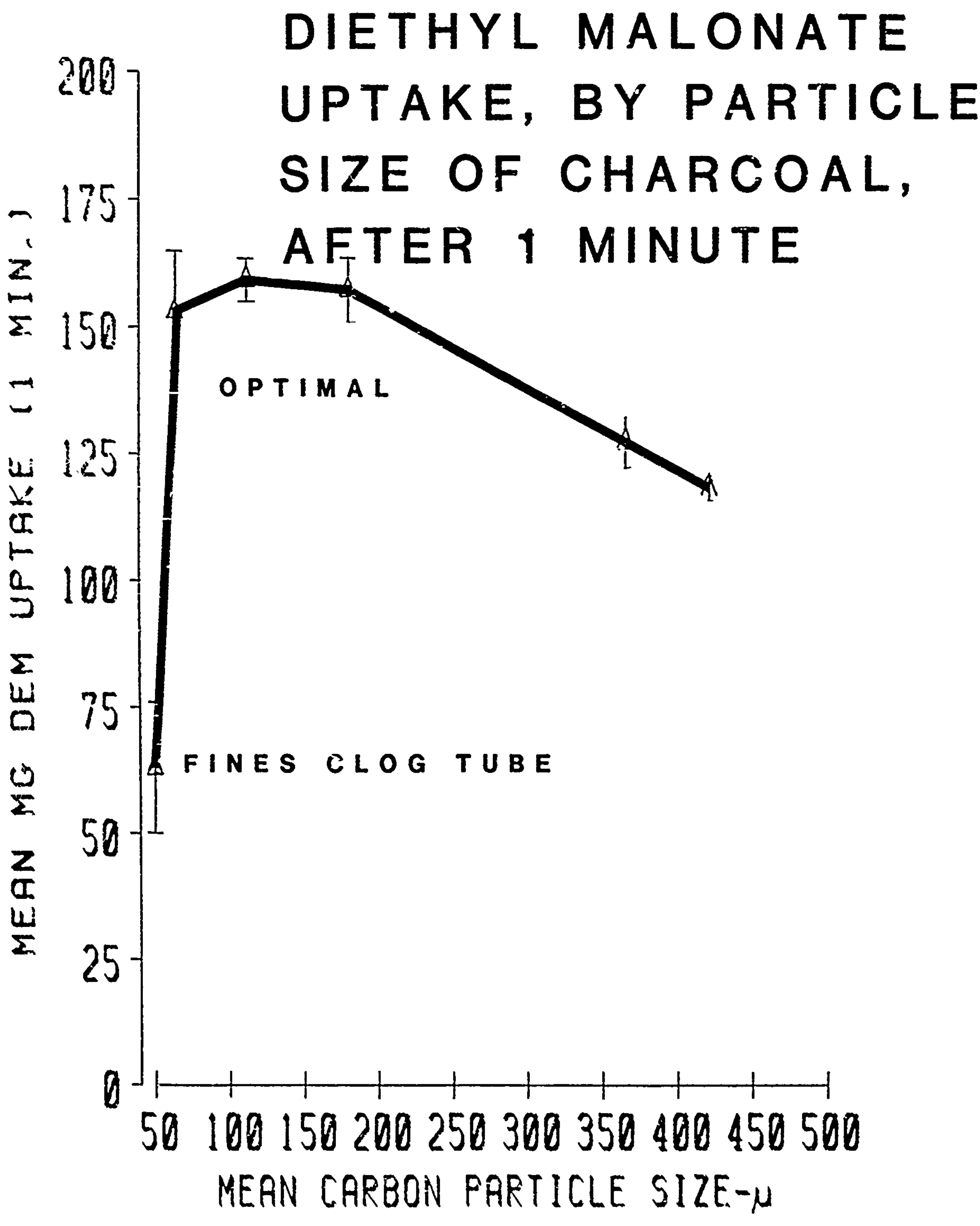

DIETHYL MALONATE BREAKTHROUGH TIME, TO M-8 PAPER ABOVE 15 MM DEEP CHARCOAL COLUMN
MEAN TIME TO BREAKTHROUGH (SEC.)
0
25
50
75
100
125
150
175
200
FINES CLOG TUBE
COARSE : FAST
50 100 150 200 250 300 350 400 450 500
MEAN CARBON PARTICLE SIZE-μ

INTERNAL SPACE OF PARTICLES

SCREENING OF TWO CLAYS AND SIX RESINS REVEALED CLAY 004 AS MOST FAVORABLY DISTRIBUTED IN THE SIZE RANGE FOUND OPTIMAL WITH CHARCOAL (GRAPH BELOW). FULLER'S EARTH HAS A WIDER DISTRIBUTION, WITH MUCH FINE MATERIAL. RESIN SIZES ARE EITHER BROADLY DISTRIBUTED OR BIPHASIC. SUCH DIFFERENCES APPEAR INSUFFICIENT TO EXPLAIN THE UPTAKE ADVANTAGE OF CLAY 004. A POSSIBLE ALTERNATE EXPLANATION IS SUPPLIED BY ANGLO AMERICAN CLAYS, INC., DEVELOPERS OF THE CLAY THEY CALL RT004. THEIR DIAGRAM, (BELOW) SHOWS INTERNAL STRATA WITH CHARGED SURFACES (NEGATIVE). THEIR PROPRIETARY METHOD FOR REMOVAL OF WATER AND COUNTER-IONS LEAVES AN EFFECTIVE INORGANIC SPONGE WITH CATALYTIC POTENTIAL (INDICATED IN OTHER DATA).



MONTMORILLIONITE / BENTONITE


DIETHYL MALONATE UPTAKE, BY PARTICLE SIZE OF CHARCOAL, AFTER 1 MINUTE
MEAN MG DEM UPTAKE (1 MIN.)
200
175
150
125
100
75
50
25
0
OPTIMAL
FINES CLOG TUBE
50 100 150 200 250 300 350 400 450 500
MEAN CARBON PARTICLE SIZE-μ

A-1012

SUMMARY

1. METHOD DISTINGUISHES BEST SORBENT
2. OPTIMAL PARTICLE SIZE RANGES EXIST
3. TEST METHOD IS TEDIOUS BUT USEFUL

A-1013

PROGRESS IN SKIN DECONTAMINATION AND PROTECTION MATERIALS

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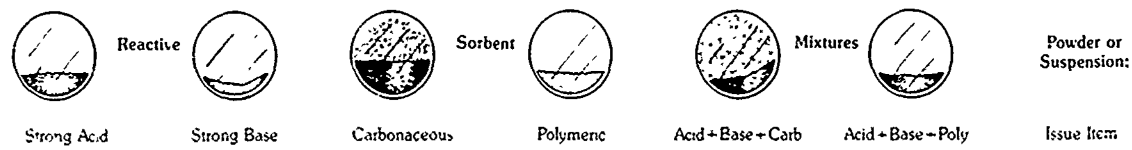
New materials are becoming available as candidates to replace existing decontaminants to prevent skin contact with toxic chemical agents. Prototype materials are displayed. Several innovative approaches have been applied in design of these products, they can be observed to differ widely in color, texture, and indicated mode of use. These prototype compositions are displayed in the context of the perceived problems that they, or future materials, will be expected to solve. Fielded materials tend to react concurrently with threat agents and with the skin to be defended. Therefore, a variety of approaches are directed toward isolating reactive groups from skin but not from contaminants. The shielded reactive sites of sorptive polymers and modified clays or catalytic matrix material are not visible, but the tangible materials are present as representative prototypes. The decontaminant reagents also serve as prototypes of possible components for future skin barrier formulations. Some of the proposed protective coatings on display may benefit from incorporation of such reagents in the future. For now, the display is intended to provide an outline of a dynamic effort that shows both progress and need for further constructive ideas.

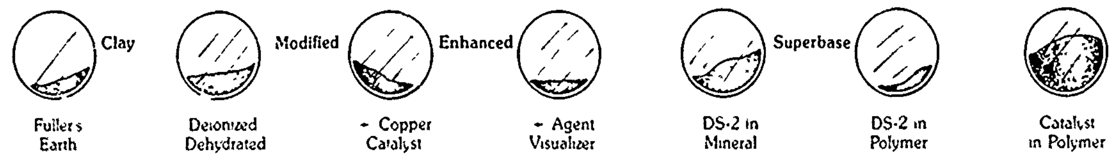
- **Problem:** Decontaminate skin without damage presently caused by ions in M258A1 solutions.
  Approach: Separate reactants from skin: sorbagent into material with reactive sites inside.

Phase I — Prototype Resins and Mixtures

Products found to be nonirritating and effective, *in vitro* and *in vivo*, against agents

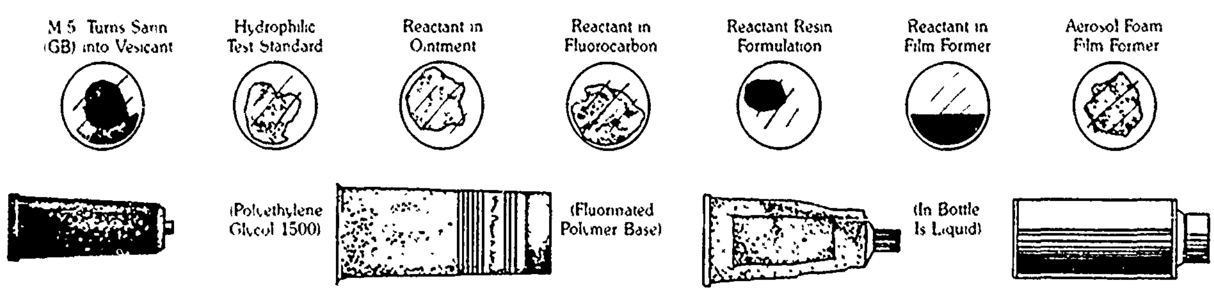


Phase II — Modified Fuller's Earth, Polymers



- **Problem:** Protect skin with effective, wearable, removable coating; unavailable.
  Approach: Investigate novel agent repellants and/or reactant formulations for protection.

Exploratory Phase: Collect Candidates



Brief Description of Candidate Materials

The reactive ions in M258A1-I solution include hydroxide, ethoxide, and phenolate. Irritant ions in M258A1-II solutions/slurries include hypochlorite and zinc (Zn^{++}). Each of these ions may damage skin structures. Prototype resin polymers are made with strongly acidic or basic groups attached to polymer strands that are buried within porous networks into which agents may diffuse and become detoxified. The carbonized or polymeric sorptive components of mixtures imbibe agents more rapidly than the reactive resins, which prevent desorption from the mixtures. The fuller's earth family of clay minerals sorb agents between negatively charged silicate plates that also sorb water and cations. Proprietary methods are used to remove as many ions and as much water as possible. Lack of competition for the charged sites may account for observed increases in both rates and volumes of agents picked up and retained by the newly modified fuller's earth derivatives. Other silicates may serve as inorganic sponges that can limit access to skin by the highly corrosive equipment decontaminant DS-2. Polymer strands may also be used to trap hydrolytic functionalities of DS-2 or catalytic structures of other materials. Proposed use of the various described decontaminant materials as components of candidate skin protectants is now being explored. Hydrophilic or fluorinated bases are inhospitable solvents for agents that may also suffer attack by incorporated reagents.

9. Sensory Effects

ELECTROPHYSIOLOGICAL CHANGES IN PERIPHERAL SENSORY RECEPTORS FOLLOWING SUB-ACUTE ADMINISTRATION OF SOMAN

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Medical College of Georgia, Augusta, Georgia

INTRODUCTION

MANY ORGANOPHOSPHOROUS AGENTS PRODUCE A DELAYED NEUROTOXICITY WHICH IS CHARACTERIZED BY ATAXIA, LOSS OF REFLEXES, AND MUSCLE WEAKNESS. SOMAN IS AN ORGANOPHOSPHOROUS AGENT WHICH IS HIGHLY REACTIVE. THE PURPOSE OF THIS STUDY WAS TWO-FOLD:

1) TO DETERMINE WHETHER SOMAN WILL PRODUCE A DELAYED NEUROTOXICITY.

2) TO DETERMINE THE EFFECTS OF SOMAN ON BOTH PROPRIOCEPTIVE AND CUTANEOUS SENSORY RECEPTOR FUNCTION REGARDLESS OF WHETHER IT DOES OR DOES NOT PRODUCE THE DELAYED NEUROTOXICITY.

THIS STUDY WAS CARRIED OUT ON MUSCLE SPINDLE PRIMARY AND SECONDARY ENDINGS AND THE FOLLOWING CUTANEOUS MECHANORECEPTORS: FIELD, RAPIDLY ADAPTING, SLOWLY ADAPTING TYPE 1, AND SLOWLY ADAPTING TYPE 2 RECEPTORS.

METHODS

CATS WERE INJECTED WITH VARIOUS DOSES OF SOMAN TO SEE IF A DELAYED NEUROTOXICITY COULD BE PRODUCED.

1. SINGLE DOSE

THE SINGLE DOSE INJECTIONS WERE 1.0 MG/KG (S.C.) AND 1.5 MG/KG (S.C.). TWENTY MINUTES PRIOR TO THE ADMINISTRATION OF SOMAN, AN INJECTION OF PHYSOSTIGMINE (1.7 MG/KG) AND ATROPINE (1.0 MG/KG) WAS ADMINISTERED I.P. THOSE ANIMALS WHICH SURVIVED WERE OBSERVED FOR UP TO 45 DAYS POST-INJECTION.

2. MULTIPLE DOSES

MULTIPLE DOSES OF SOMAN WERE ADMINISTERED OVER VARIOUS TIME PERIODS. THE DOSING SCHEDULES WERE AS FOLLOWS:

A. 5 UG/KG/DAY FOR FIVE DAYS (TOTAL OF 25 UG/KG)

B. 2.5 UG/KG/DAY FOR 10 DAYS (TOTAL OF 25 UG/KG)

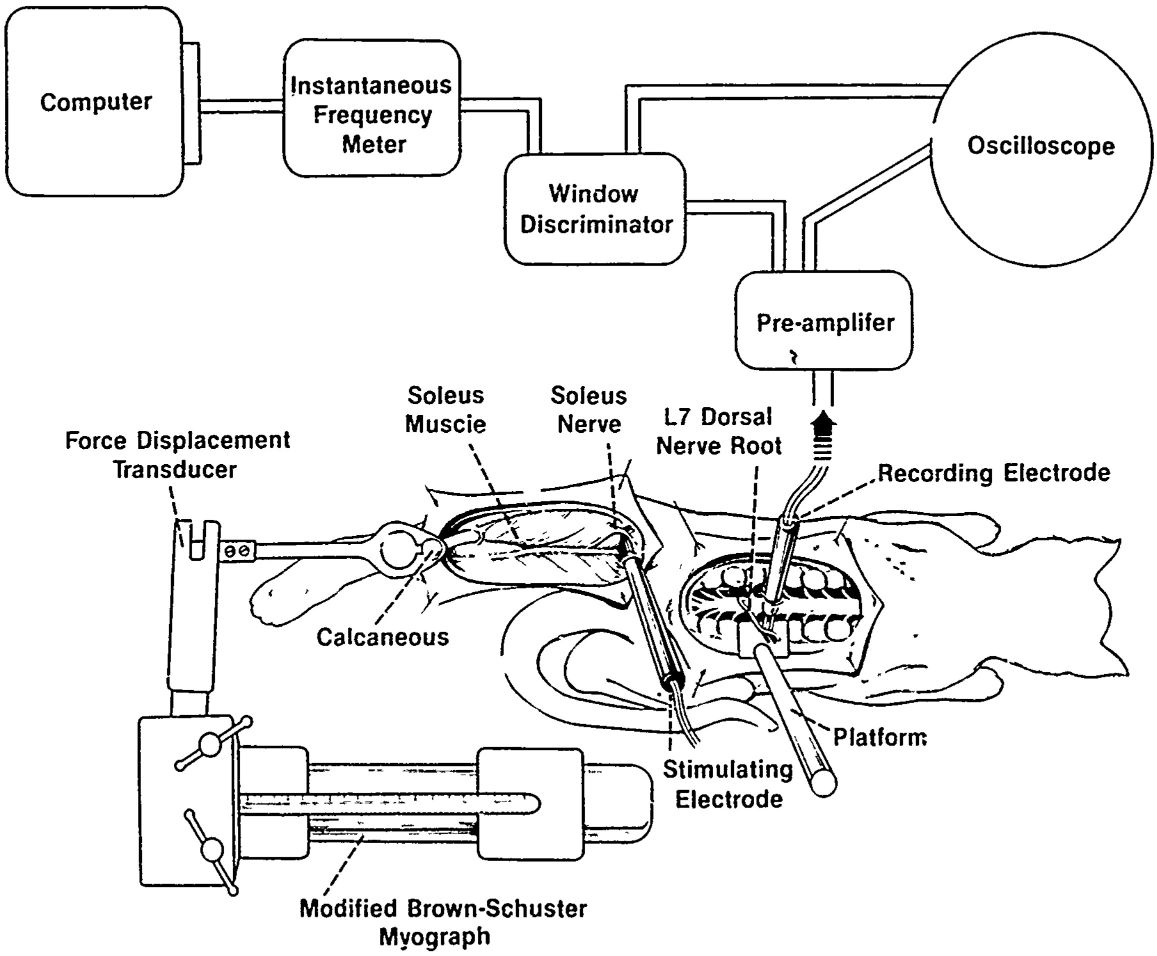
THE EXPERIMENTS ON SENSORY RECEPTOR FUNCTION WERE PERFORMED ONE DAY FOLLOWING THE LAST INJECTION. THESE STUDIES INCLUDED THE TESTING OF MUSCLE SPINDLE FUNCTION AND CUTANEOUS TACTILE RECEPTOR FUNCTION.

STUDIES ON CUTANEOUS TACTILE RECEPTORS WERE CARRIED OUT AS DESCRIBED IN THE PREVIOUS POSTER. MUSCLE SPINDLE PRIMARY AND SECONDARY ENDINGS WERE RECORDED FROM THE SOLEUS MUSCLE. BRIEFLY, A LAMINECTOMY WAS PERFORMED. THE L_7 AND S_1 SPINAL ROOTS WERE ISOLATED AND CUT PROXIMAL TO WHERE THEY ENTER THE CORD. THE SOLEUS MUSCLE AND NERVE WERE ISOLATED AND THE REST OF THE LEG WAS DEAFFERENTED. FOR THE REST OF THE RECORDING SET-UP SEE THE ADJOINING FIGURE.

FREQUENCY-RESPONSE CURVES WERE GENERATED AND RECEPTOR COUNTS WERE PERFORMED ON THE TACTILE RECEPTORS.

NONE OF THE DOSAGE REGIMENS DESCRIBED ABOVE PRODUCED
ANY OVERT SIGNS OF A DELAYED NEUROTOXICITY

RECORDING SET-UP FOR MUSCLE SPINDLES



RECORDING SET-UP FOR TACTILE RECEPTORS

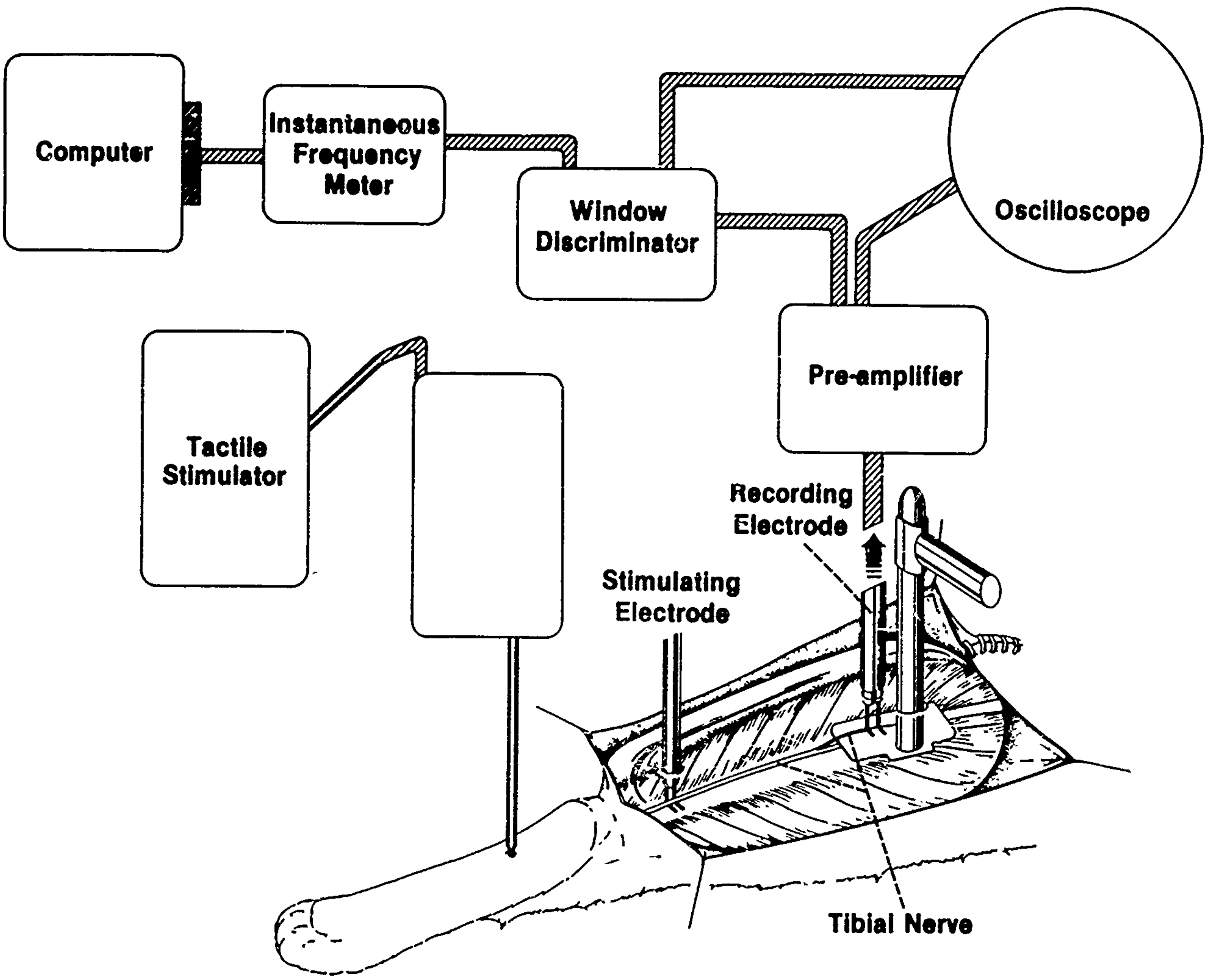
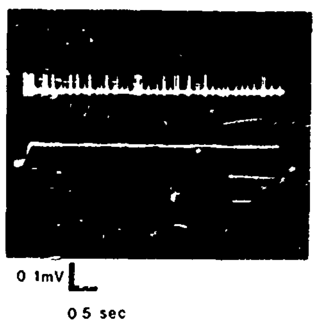
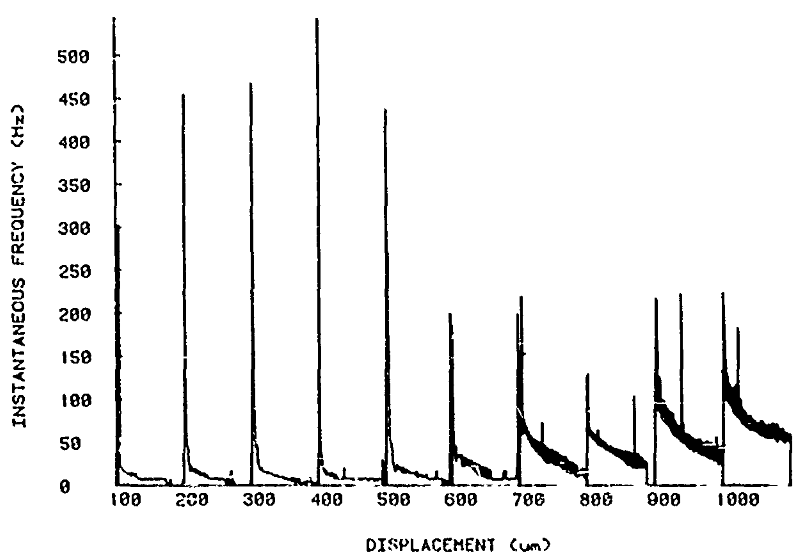


TABLE 1. CRITERIA FOR IDENTIFICATION OF CUTANEOUS MECHANORECEPTORS

MECHANORECEPTOR TYPE	TYPE OF RESPONSE	GENERAL RECEPTIVE FIELD
FIELD (F)	VELOCITY ONLY	WIDESPREAD
RAPIDLY ADAPTING (RA)	VELOCITY ONLY	PUNCTATE
SLOWLY ADAPTING TYPE 1 (SA1)	VELOCITY AND PROLONGED DISPLACEMENT; IRREGULAR DISCHARGE	PUNCTATE
SLOW ADAPTING TYPE 2 (SA2)	VELOCITY AND PROLONGED DISPLACEMENT; REGULAR DISCHARGE; RESPONDS TO STRETCH	PUNCTATE

SLOWLY ADAPTING RECEPTOR DISCHARGE





Time Interval = 9.95 secs

FIGURE 1. SOMAN HAS NO EFFECT ON MUSCLE SPINDLE PRIMARY ENDINGS

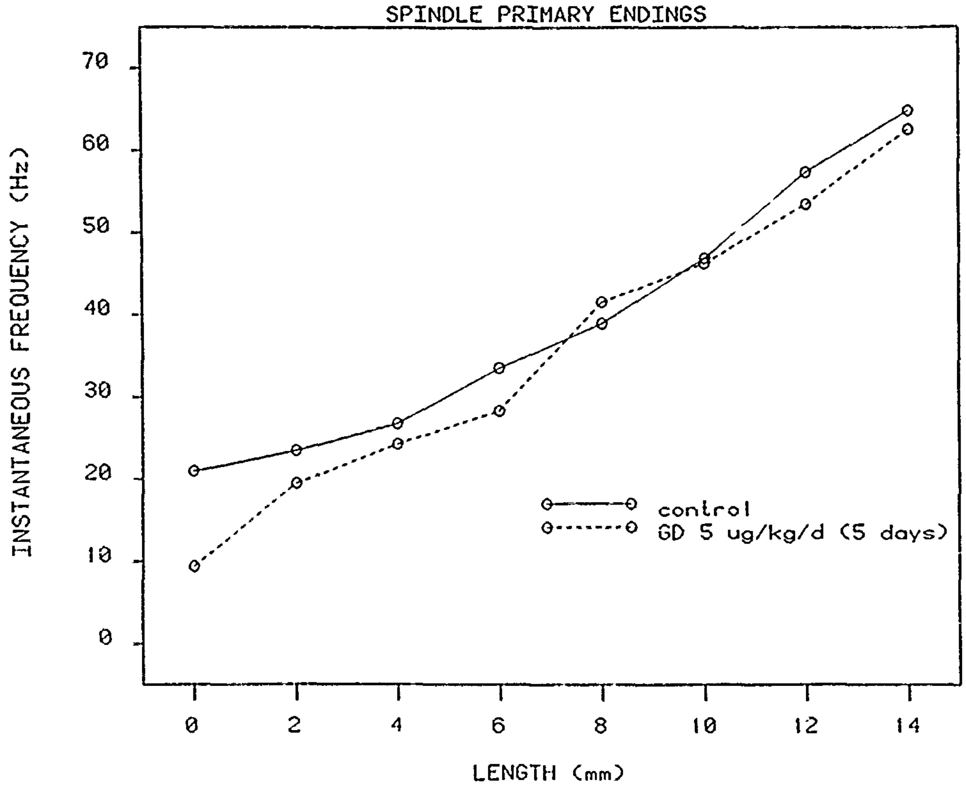


FIGURE 2. SOMAN HAS NO EFFECT ON MUSCLE SPINDLE SECONDARY ENDINGS

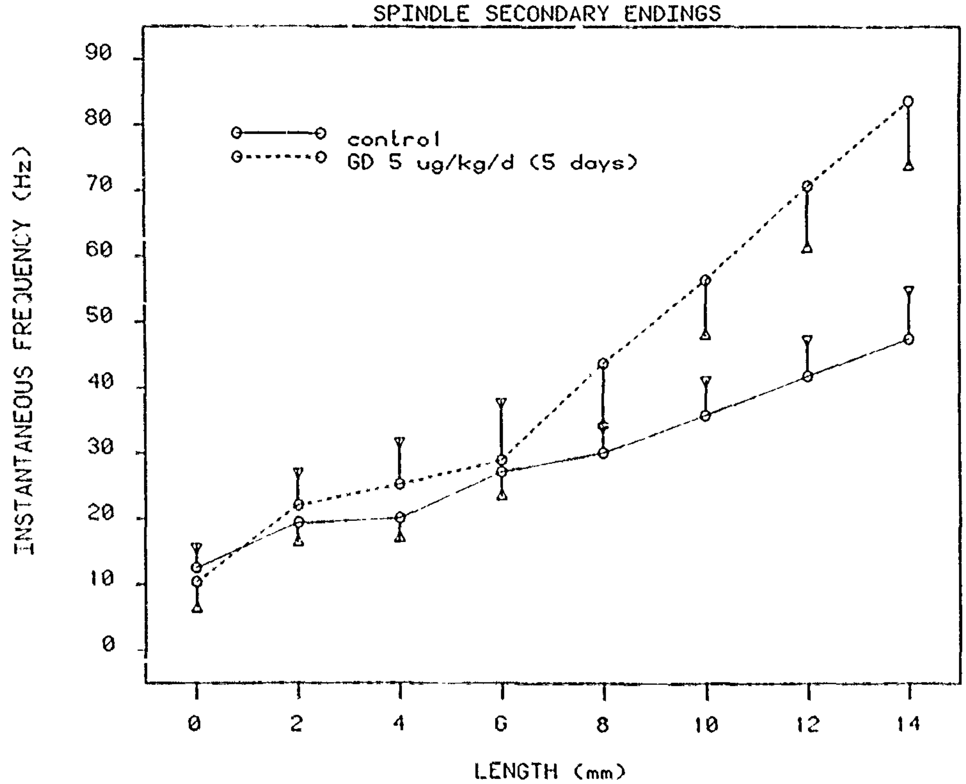


FIGURE 3. SOMAN HAS NO EFFECT ON THE FREQUENCY RESPONSE OF TYPE SA1 MECHANORECEPTORS

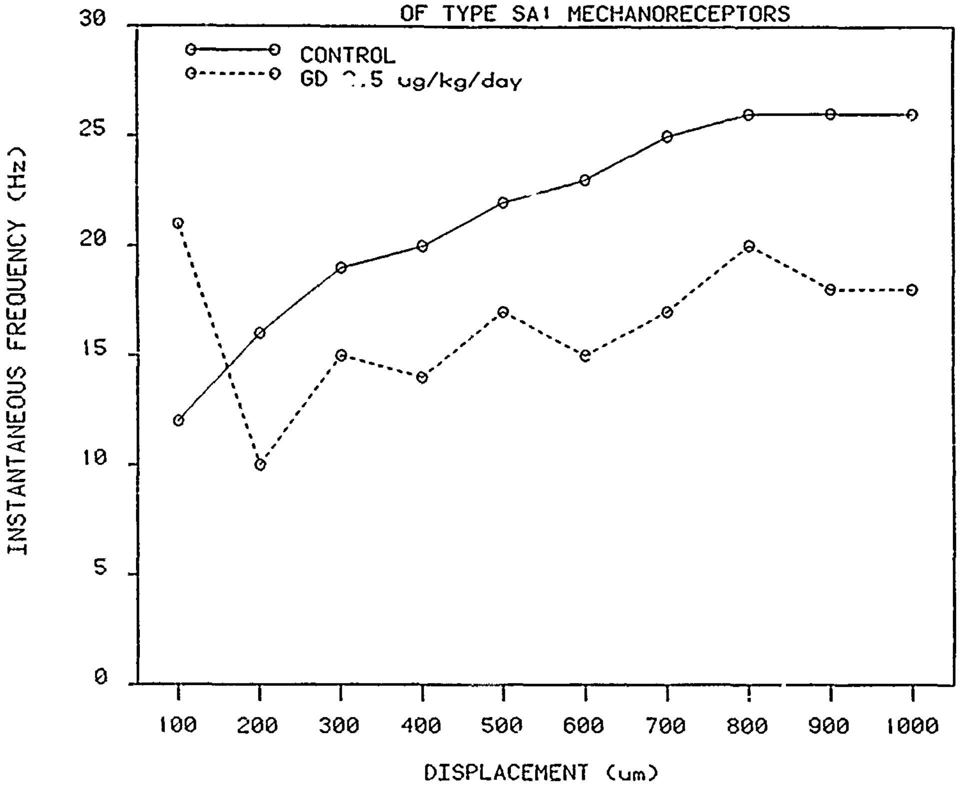
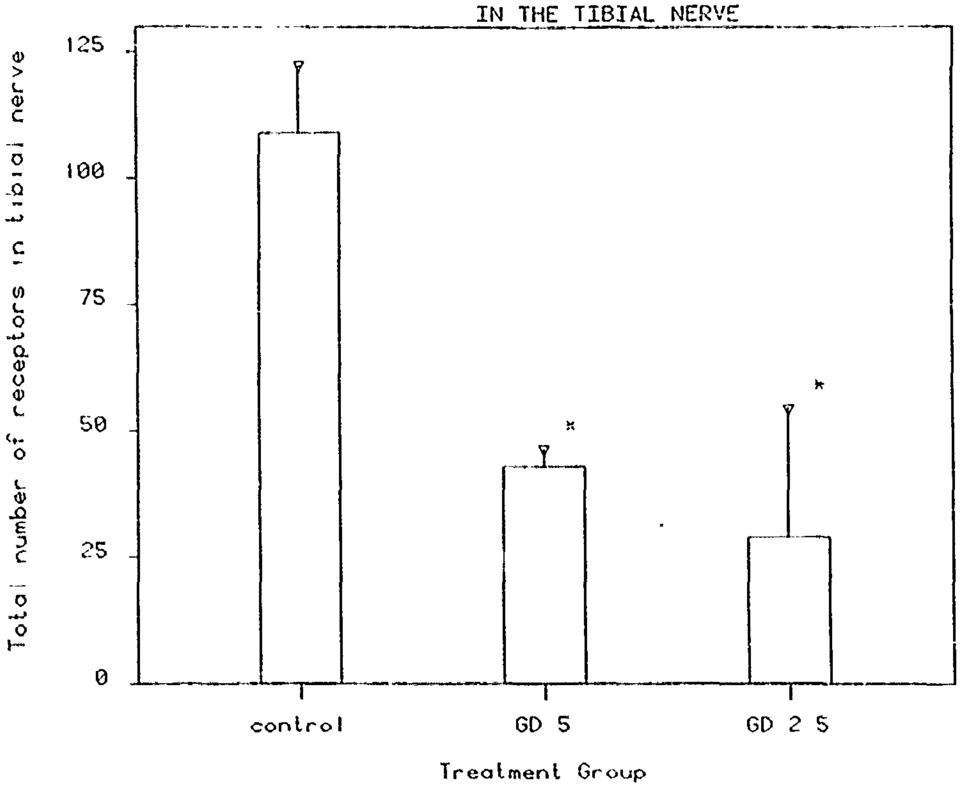


FIGURE 4 SOMAN REDUCES THE TOTAL NUMBER OF RECEPTORS IN THE TIBIAL NERVE



SOMAN HAS LITTLE OR NO EFFECT ON THE FREQUENCY DISTRIBUTION OF MECHANORECEPTORS

TABLE 1

FREQUENCY DISTRIBUTION OF RECEPTORS TYPES [1]

	CONTROL [2,3]	2.5 UG/KG	5 UG/KG
FIELD	63±1.8	56±1.2	64±5
RAPIDLY ADAPTING	15±1.8	22±4.5	23±3.6
SLOWLY ADAPTING TYPE 1	17±1.5	13±2.7	12±3.8
SLOWLY ADAPTING TYPE 2	5±0.8	9±0.7	1±1.2

[1] PERCENT OF TOTAL RECEPTORS OBTAINED PER FASICLE

[2] MEAN ± SEM

[3] NUMBER OF ANIMALS IS 5 IN CONTROL AND 3 EACH IN THE TREATED GROUPS

CONCLUSIONS

1. SOMAN DOES NOT PRODUCE A DELAYED NEUROTOXICITY

2. SUB-ACUTE ADMINISTRATION OF SOMAN DOES NOT AFFECT PROPRIOCEPTIVE FUNCTION

3. SUB-ACUTE ADMINISTRATION OF SOMAN DOES NOT AFFECT THE FREQUENCY RESPONSE OF SLOWLY ADAPTING TYPE 1 MECHANO-RECEPTORS

4. SUB-ACUTE ADMINISTRATION OF SOMAN DOES REDUCE THE TOTAL NUMBER OF TACTILE RECEPTORS IN THE TIBIAL NERVE

5. THE EXACT REASON FOR A PREFERENTIAL EFFECT OF SOMAN ON CUTANEOUS MECHANORECEPTORS IS UNCLEAR.

THE EFFECTS OF A CARBAMATE AND AN ORGANOPHOSPHATE ON THE CAT VISUAL SYSTEM

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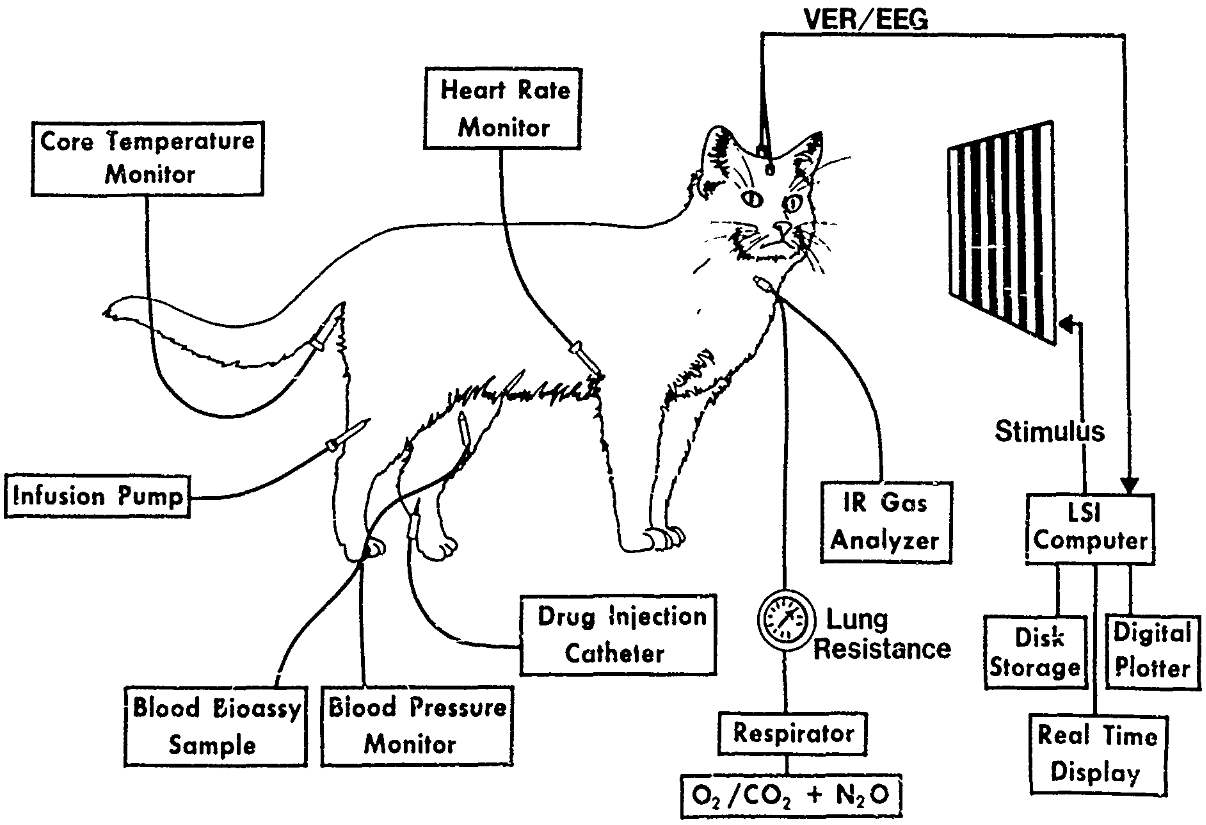
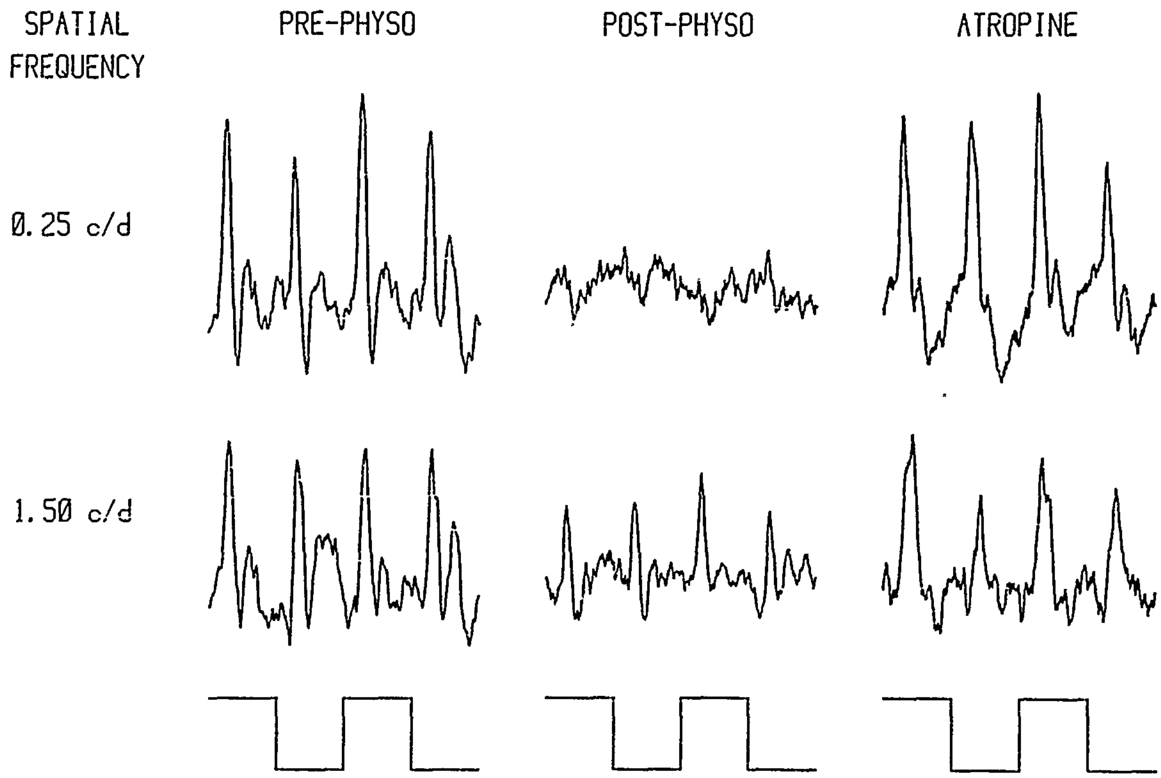
INTRODUCTION

Cholinergic influences have been found at various stages of processing within the primary visual pathway. In the cat, indirect evidence has been found for cholinergic neurons in the retina, lateral geniculate nucleus and visual cortex. Inhibition of acetylcholinesterase (AChE), thus preventing the hydrolysis of acetylcholine (ACh), should cause transmission losses along the visual pathway. To study these losses, we recorded visual evoked responses (VERs) from Area 17 before and following administration of physostigmine sulfate (a reversible inhibitor of AChE) and diisopropyl fluorophosphate (DFP; an irreversible inhibitor of AChE).

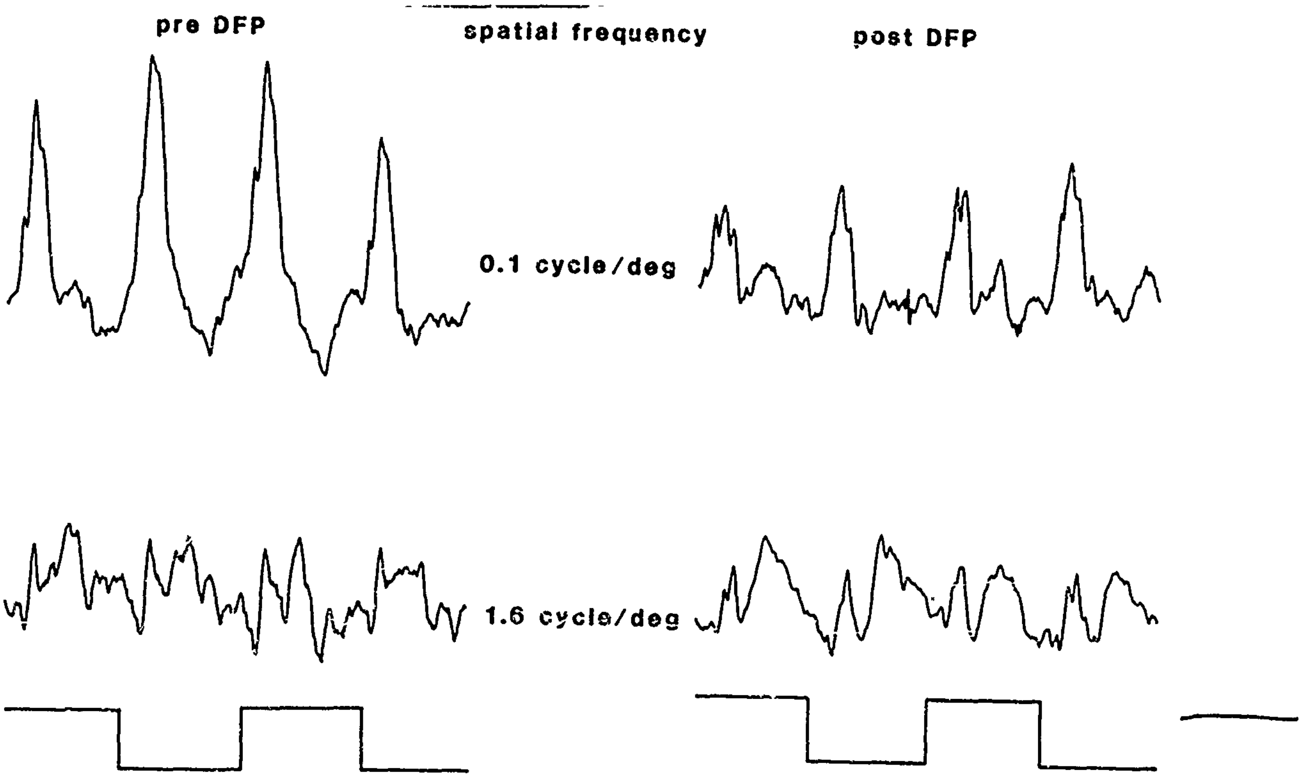
PREPARATION



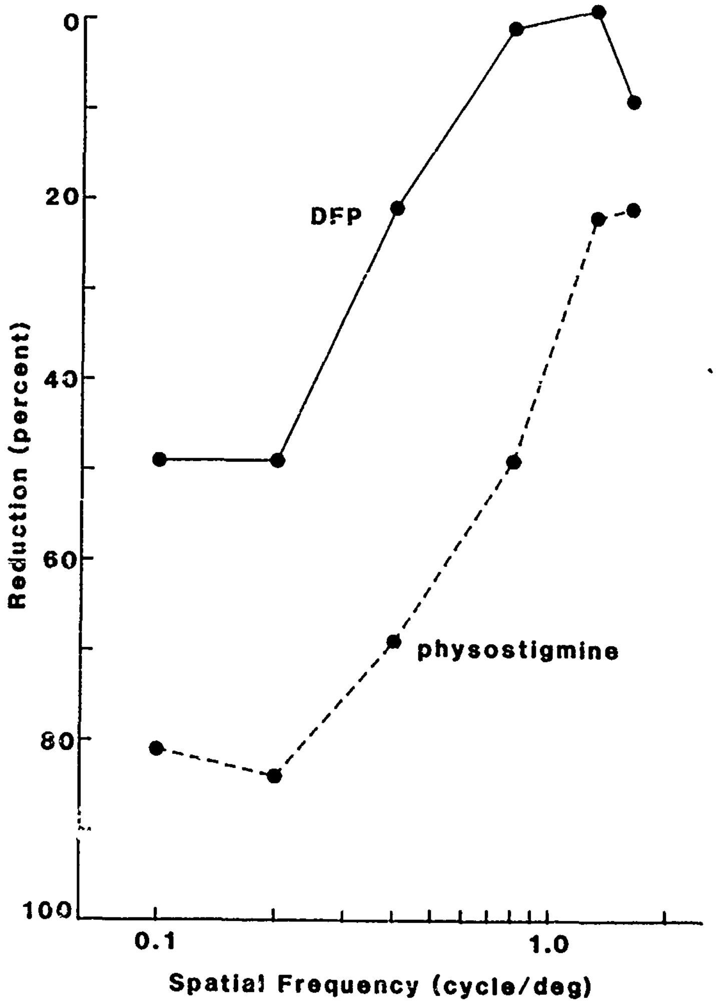
Anesthetized and paralyzed adult cats were held in a stereotaxic headholder and fitted with appropriate corneal and auxiliary lenses to focus one eye onto a cathode ray tube (CRT). The other eye was occluded. Square wave luminance gratings were generated on the face of the CRT and phase alternated at 2 Hz. VERs were recorded with stainless steel bone screws over the visual and parietal cortex. Stimulus presentation and collection of response histograms were controlled by computer. Arterial blood samples were used to measure blood gases as well as AChE levels. Following determination of the baseline response and initial enzyme levels, physostigmine (0.5 mg/kg) or DFP (0.5 to 4 mg/kg) was administered IV over one minute. VERs and enzyme levels then were measured periodically.



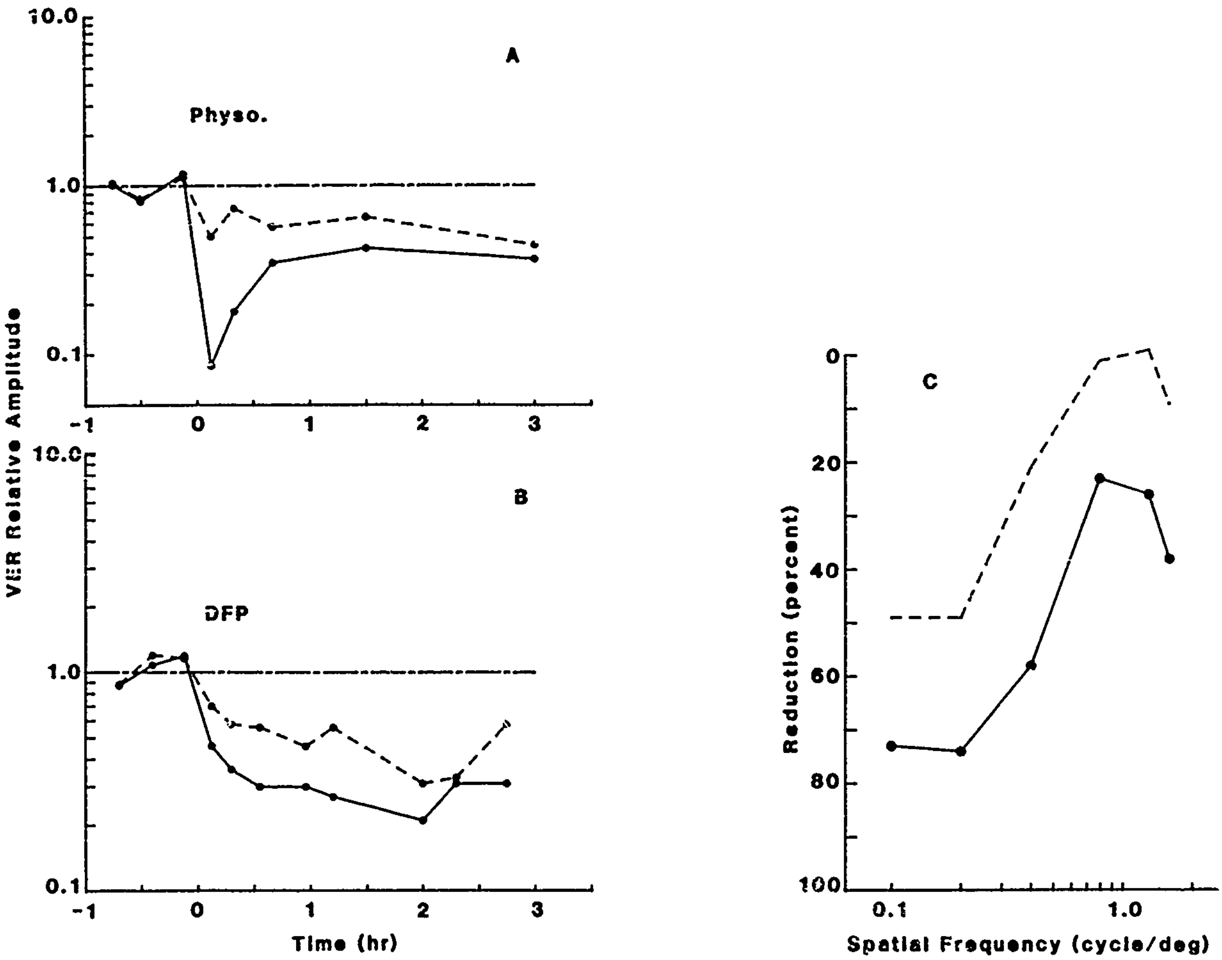
VER averages (120-second collection period with a 1-msec sampling interval) for two spatial frequencies from a single experiment. (A) Baseline VERs. (B) VERs after injection of physostigmine (0.5 mg/kg). (C) Recovery VER's after injection of atropine sulphate (0.5 mg/kg). Bottom row depicts the 2-Hz square-wave alternation of the grating pattern. Contrast = 0.40.



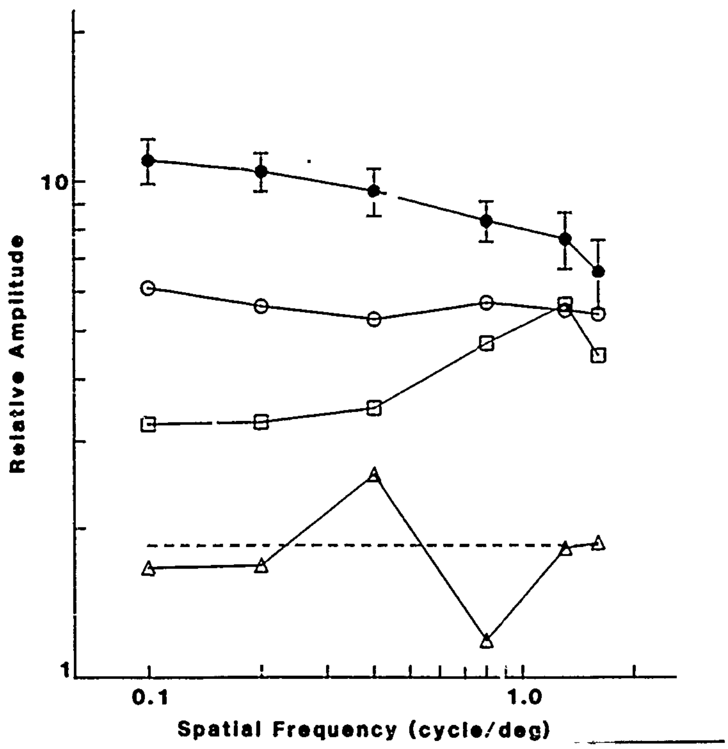
VER averages, from a single experiment, to a low and high spatial frequency prior to and following 5.0 mg/kg DFP. The visual effects of DFP could also be reversed by atropine sulphate (data not shown). Grating contrast = 0.40.



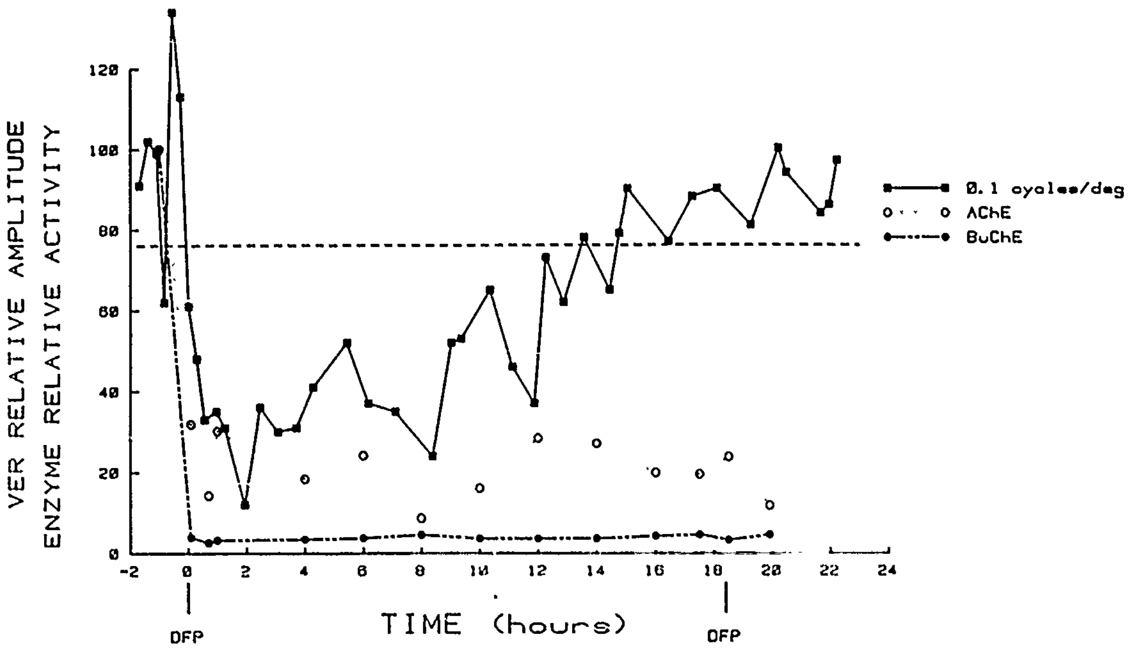
Averaged data showing VER reduction for different spatial frequencies from 5 cats receiving 4.0 mg/kg DFP and 5 cats receiving 0.5 mg/kg physostigmine. Data obtained from first collection period immediately following drug administration. Note the similar reduction to low spatial frequencies. Although physostigmine caused greater visual losses, it reduced blood AChE by an average of only 46% compared to an average 83% reduction to DFP. Blood AChE measured 5 min post administration.



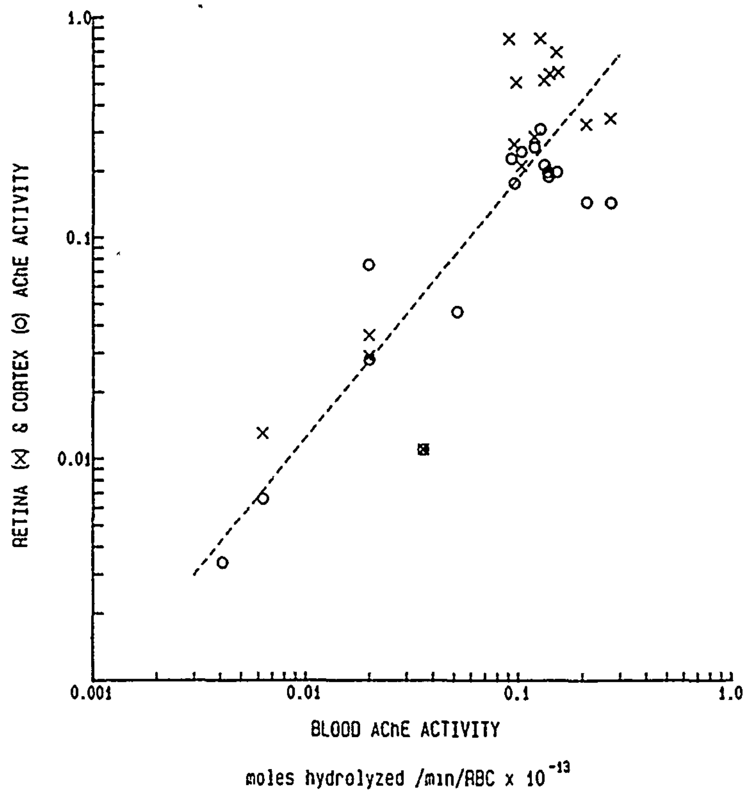
Averaged VER amplitudes to low spatial frequencies (solid line) and to high spatial frequencies (dashed line) as a function of time before and following 0.5 mg/kg physostigmine (A) and 4 mg/kg DFP (B). Baseline responses were set equal to 1.0 (broken line). (C) Average of the maximum reduction in VER amplitude for each spatial frequency following 4.0 mg/kg DFP for the same cats as in the preceding figure. The DFP data from the preceding figure showing initial reduction are replotted (dashed curve). Grating contrast = 0.40.



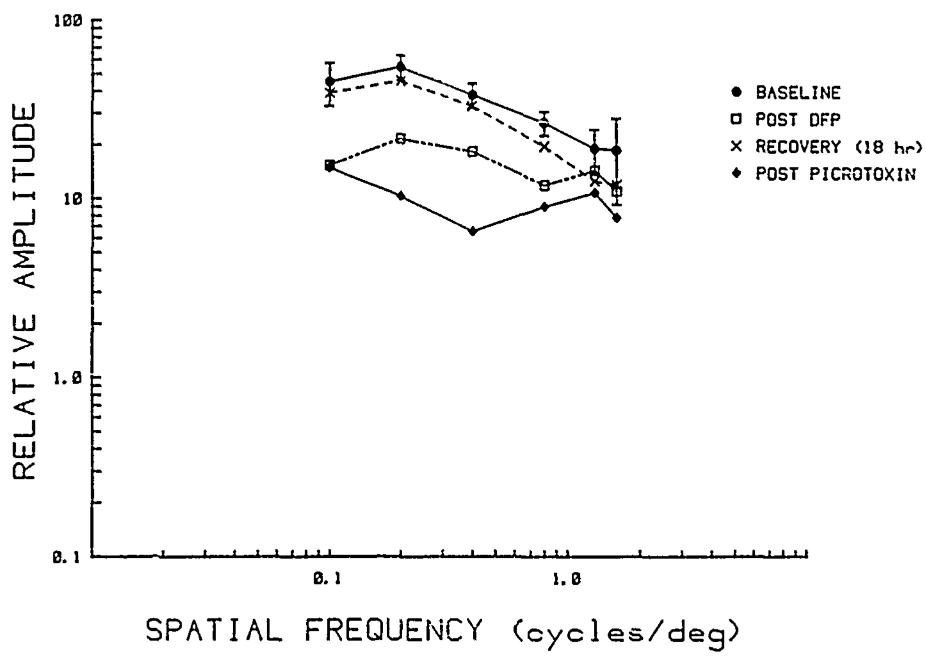
Relative amplitudes of the VER signal to different spatial frequencies from a single experiment. Filled circles represent the average of 5 baseline measures showing ±1 S.D. The remaining curves (from top to bottom) represent average VER signals to 1 mg/kg, 2 mg/kg and 4 mg/kg DFP respectively. Doses of DFP were given two hours apart. The dashed line represents the measured noise level. Note that at 4 mg/kg the VERs were completely abolished at all spatial frequencies. Grating contrast = 0.40.



Reduction and recovery to baseline levels of the VER to a single low spatial frequency grating following 4 mg/kg DFP given at time 0. Although the VER recovered in about 14-16 hours, there was no recovery in blood AChE or BuChE activity. At time 18.4 hours, a second dose of 4 mg/kg DFP was administered with no discernable visual effect. This tolerance suggests that conditions responsible for visual recovery are not identical to baseline conditions. Dashed horizontal line represents a -1 S.D. for six baseline responses.



Correlation of blood AChE levels with retina and visual cortex AChE levels. Data points clumped together at right represent normal control values. Depressed data values at left represent those following DFP.



VER reduction following 4 mg/kg DFP, and recovery in about 18 hours. Administration of 0.3 mg/kg picrotoxin, an antagonist of gamma-aminobutyric acid, then reduced the VER in a manner similar to cholinesterase inhibitors suggesting a GABA compensation role. Supportive preliminary data from HPLC analysis shows an average 3-fold increase in GABA in visual cortex six hours following 4 mg/kg DFP.

CONCLUSIONS

1. Both DFP and physostigmine reduced the VER to low spatial frequencies more than high, supporting a multi-channel model of spatial frequency responsivity.

2. The effect is cholinergic since it could be reversed by atropine.

3. Although DFP caused greater reduction of blood AChE than did physostigmine, DFPs visual effect was smaller.

4. Although the low spatial frequency response is cholinergic-sensitive, high doses of DFP abolish responses to all spatial frequencies.

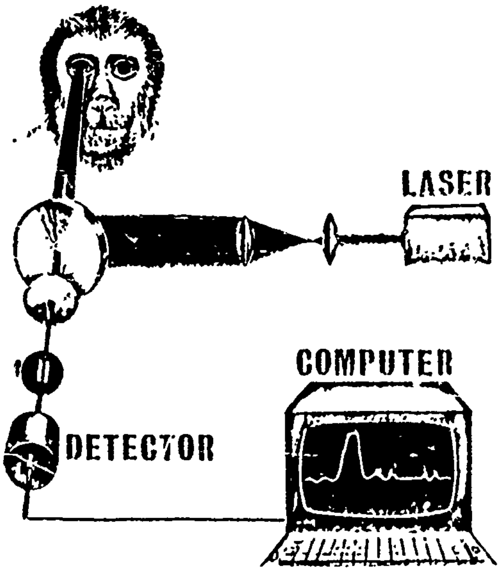
5. Spontaneous recovery of the VER without a concomitant recovery of AChE suggests that compensatory mechanisms are controlling ACh levels. GABA compensation appears likely.

6. DFP tolerance appears linked to compensatory mechanisms and a depression of AChE.

RESEARCH FOR THE SOLDIER

EFFECTS OF (3,3-DIMETHYL-2-BUTOXY)-METHYL-PHOSPHORYLFLUORIDE (SOMAN) ON THE IMAGING PROPERTIES OF THE PHYSIOLOGICAL OPTICS IN *MACACA MULATTA*

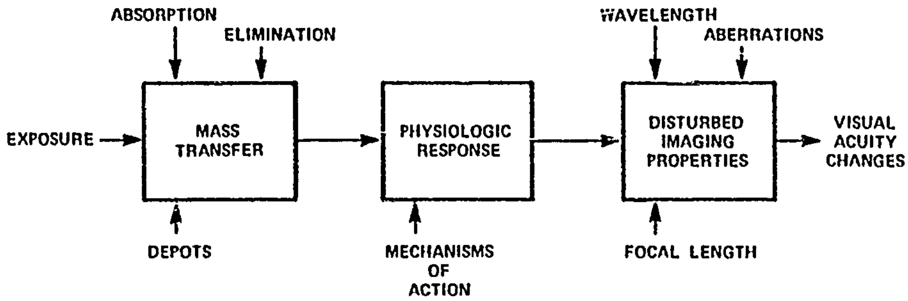
G.D. Polhamus, D.K. Cohoon, M.D. Green and P.C. Jones
US Army Medical Research Institute of Chemical Defense
Aberdeen Proving Ground, Maryland 21010-5425

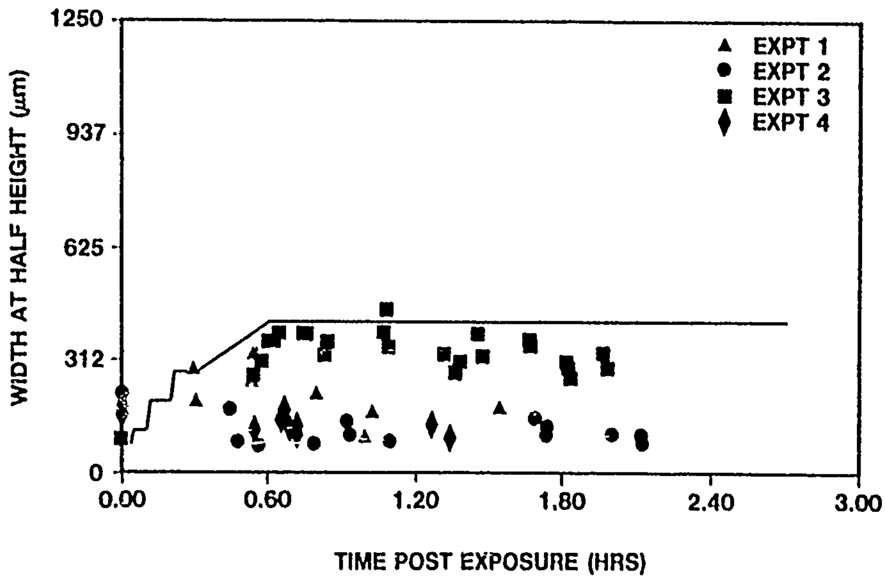


One of the first effects of exposure to nerve agents is disturbed visual function. Nerve agent vapor is rapidly absorbed into the tear layer of the eye and diffuses through the cornea into the anterior chamber, causing miosis and accommodative spasm. We instilled 0.14, 1.4 & 4.2 mg of soman diluted in 20 ml of saline into the conjunctival sac of M. Mulatta and measured the effects on the physiological optics in vivo by photometrically analyzing the image created by a 647 nm laser beam, which was reflected from the retina. The image was passed through a slit to produce a line spread function (LSF). The LSF is commonly used to describe the quality of optical systems and can be directly related to visual acuity and other measures of visual performance. In general, as the quality of an optical system degrades, the width of the LSF increases. Results indicate that the lowest dose of soman (0.14 mg)does not cause impairment, though progressively higher doses ($\geq$ 1.4 mg) do. The average ratio of the half power width of the LSF measured at 1 hr post-exposure divided by the pre-exposure value (and standard deviation, SD, relative to the mean) was 1.0 (SD = 30%), 2.2 (SD = 46%) & 10.9 (SD = 56%) for 0.14, 1.4, & 4.2 mg of soman respectively. Experimental measurements were compared with predictions derived from a mathematical model of both the pharmacodynamic and the imaging properties of the eye. The experimental measurements represent profound disturbances in optical quality, resulting from corneal exposures to nerve agent. Furthermore, they emphasize the particular susceptibility of young subjects with large accommodative ranges.

PROCEDURE

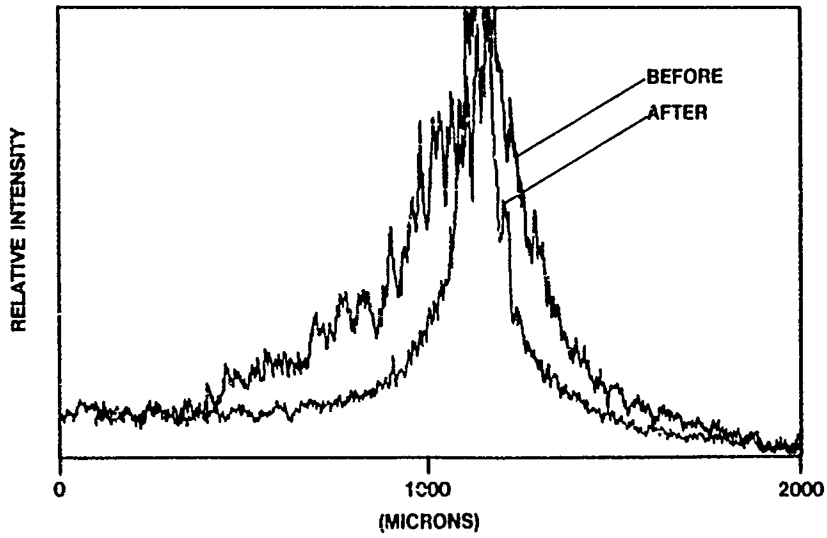
- RHESUS
  - KNOWN AGE
  - ACCOMMODATIVE RANGE MEASURED UNDER KETAMINE WITH PHENLYEPHRINE & CARBACHOL
- ANESTHESIA
  - HALOTHANE & NO_2
  - FLAXEDIL RETROBULBAR
- GD
  - 0.14, 1.4, 4.2 μg IN "TEAR" SOLUTION
  - 20 μL IN CONJUNCTIVAL SAC
- MEASURED LINE SPREAD FUNCTION OF RETINAL REFLECTION





PREDICTED AND MEASURED WIDTH AT HALF HEIGHT OF THE LINE SPREAD FUNCTION FOR 0.14 μg

MEASURED LINE SPREAD FUNCTION BEFORE AND AFTER EXPOSURE



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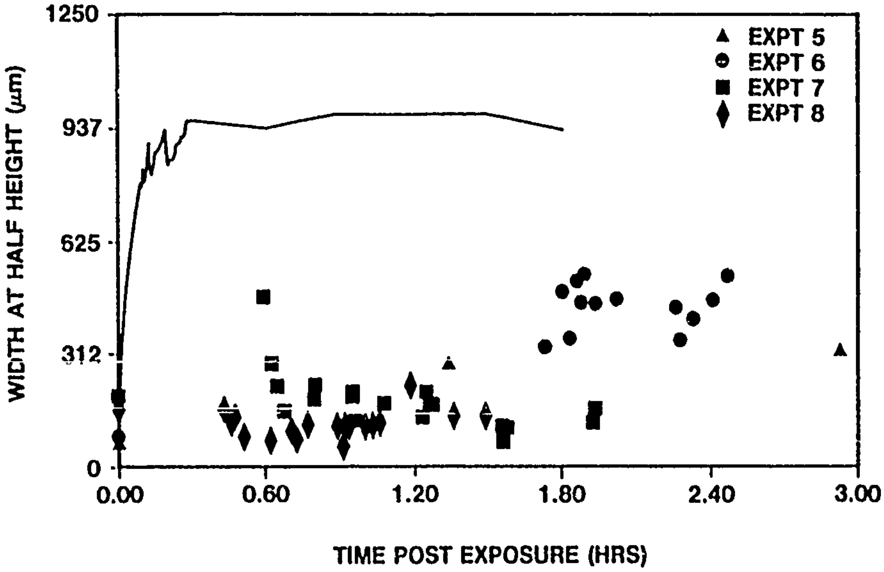
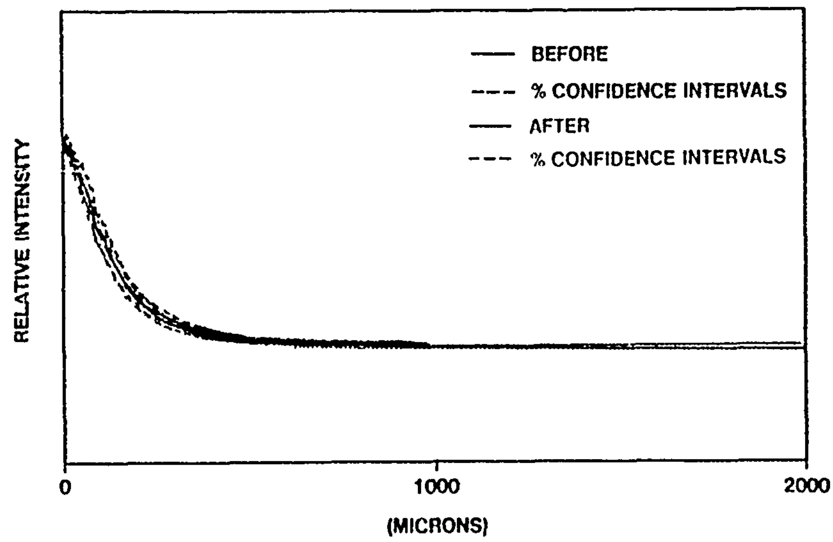
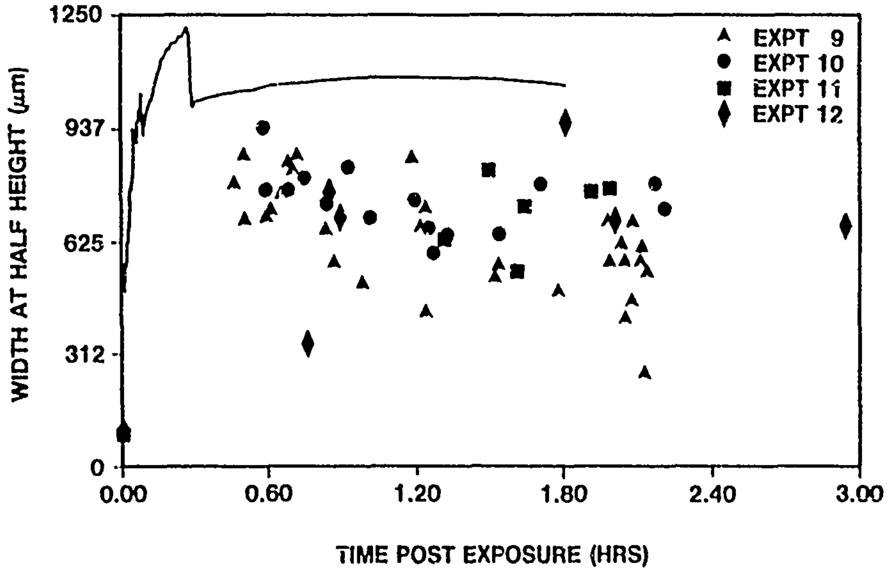


PREDICTED AND MEASURED WIDTH AT HALF HEIGHT OF LINE SPREAD FUNCTION FOR 1.4 μg

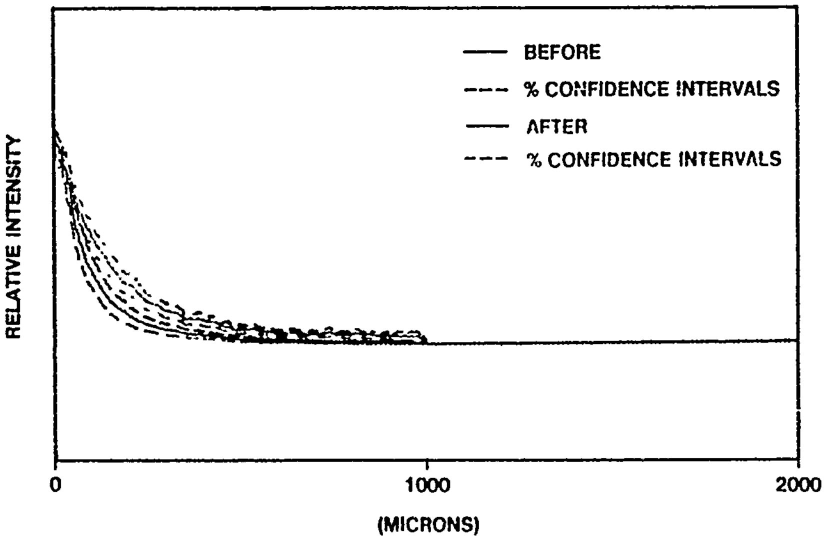
MEAN & 95% CONFIDENCE INTERVALS OF MEASURED LINE SPREAD FUNCTIONS
BEFORE AND AFTER 0.14 μg GD



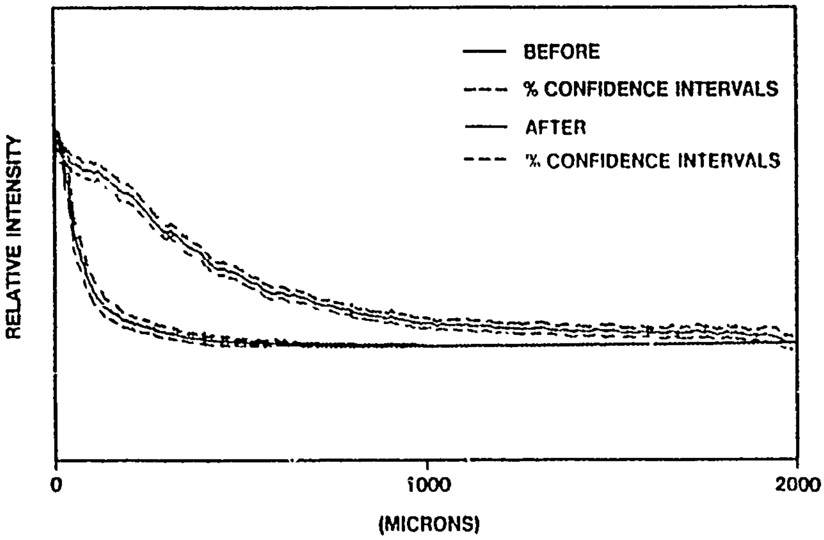


PREDICTED AND MEASURED WIDTH AT HALF HEIGHT OF
THE LINE SPREAD FUNCTION FOR 4.2 μg

MEAN & 95% CONFIDENCE INTERVALS OF MEASURED LINE SPREAD FUNCTIONS
BEFORE AND AFTER 1.4 μg GD



MEAN & 95% CONFIDENCE INTERVALS OF MEASURED LINE SPREAD FUNCTIONS
BEFORE AND AFTER 4.2 μg GD



CONCLUSIONS

- CAN MEASURE LSF DURING MIOSIS & ACCOMMODATIVE SPASM
- PREDICTIONS & MEASUREMENTS REFLECT SIMILAR TRENDS
- PRODUCT -- MODEL OF IMAGE DEGRADATION FROM ANY DOSE

ACKNOWLEDGEMENTS

ROBERT SWEENEY, PH.D.
MS. MAN SZE
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MR. FRANK S. HOVATTER

EFFECTS OF SOMAN ON THE AUDITORY BRAINSTEM RESPONSE IN GUINEA PIGS

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INTRODUCTION

THE PURPOSE OF THIS STUDY WAS TO DETERMINE THE EFFECTS OF SOMAN (GD) ON A SENSORY EVOKED RESPONSE: THE AUDITORY BRAINSTEM RESPONSE (ABR). THE ABR IS A MEASURE OF THE ELECTRICAL ACTIVITY GENERATED BY SUBCORTICAL STRUCTURES OF THE AUDITORY SYSTEM FROM THE EIGHTH NERVE TO THE INFERIOR COLLICULUS. THE COMPOSITE ABR IS A SERIES OF FIVE 'PEAKS' OR 'WAVES' GENERATED BY SUCCESSIVE AUDITORY NUCLEI (Figure 1.).

THE EFFECTS OF ORGANOPHOSPHOROUS AGENTS ON THE ABR HAVE NOT BEEN PREVIOUSLY REPORTED. PREVIOUS STUDIES HAVE SHOWN THAT CHOLINERGIC DRUGS ADVERSELY AFFECTED THE ABR (BHARGAVA, 1978), HOWEVER, THESE RESULTS ARE NOT DEFINITIVE BECAUSE THE ANIMALS WERE SEDATED AND THE TEMPERATURE OF THE ANIMAL WAS NOT MONITORED.

THE TEMPERATURE OF THE ANIMAL AT THE TIME OF RECORDING THE ABR IS CRITICAL FOR TWO REASONS: (1) THE LATENCIES OF THE ABR WAVES ARE HIGHLY CORRELATED WITH TEMPERATURE (MARSHALL & DONCHIN, 1981), AND (2) ANTICHOLINESTERASES INDUCE HYPOTHERMIA IN ANIMALS (MEETER, WOLTHUIS AND VAN BETHEM, 1971).

THERE WERE TWO PHASES IN THIS STUDY: *FIRST* WE ESTABLISHED NORMATIVE TEMPERATURE VS. LATENCY DATA. *SECOND,* WE RECORDED THE ABR FOLLOWING INJECTION OF ONE LD-50 (28 MICROGRAMS/KG, S.C.) OF SOMAN THIRTY MINUTES PRIOR TO THE SOMAN, EACH ANIMAL RECEIVED A PROTECTIVE DOSE OF ATROPINE METHYL NITRATE (16 MG/KG, I.M.).

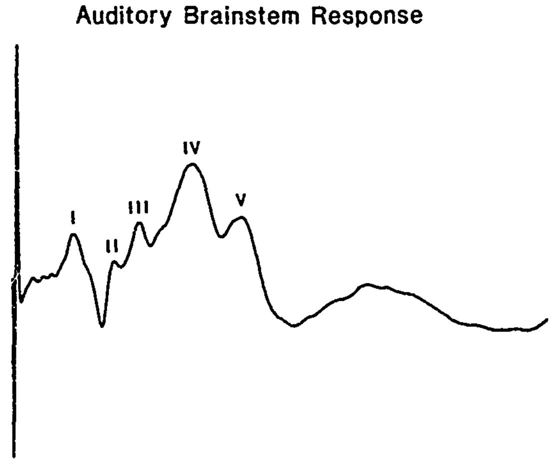


Figure 1. Auditory Brainstem Response (ABR) recorded in unanesthetized, restrained guinea pig representing an averaged response to 512 click stimuli presented at 11/second, 104 dB SPL. Presumed generators:

I - auditory nerve
II - cochlear nucleus
III - superior olive
IV - lateral lemniscus
V - inferior colliculus.

Figure 2. Guinea pig in plexiglas restrainer. Note the EEG electrode wires, YSI temperature probe and mini-speaker mounted on the animal's electrode pedestal.

METHODS

I - ANIMAL PREPARATION

** six male guinea pigs (300-400 grams)
** three stainless steel electrodes implanted over the cerebral cortex contralateral to test ear at least 5 days prior to the experiment

anterior = bregma + 6mm; 1mm lateral to midline
middle = bregma + 1mm; 1mm lateral to midline
posterior = lambda - 1mm; 1mm lateral to midline

** YSI 511 temperature thermistor placed s.c. in head region
** mini-speaker affixed to electrode pedestal

II - RECORDING

** ABR EEG bandpass = 100 Hz to 3,000 Hz
** ABR EEG amplification = 10,000 - 20,000 times
** digitizing rate = 100,000 Hz (10 microsecond dwell)
** recording epoch = 10.24 miliseconds

III - STIMULUS

** 100 microsecond click, single polarity
** stimulus rate = 11 clicks per second
** each ABR represents an average of 512 or 1024 stimulus presentations
** stimulus intensities = 104, 84 or 64 dB SPL
** monaural (left ear) stimulation

IV - EXPERIMENTAL

** Recording conditions:

restrained (Figure 2.)
body temperature continuously monitored (.01 C)
room temperature continuously monitored (.1 F)
EEG monitored continuously and stored on FM tape
ABR recorded at approximately 5 minute intervals

** Phase I - The Effect of Temperature on ABR Latency:

ABR recordings were obtained over a five degree temperature range at whole degree increments: i.e., normothermia plus one degree to normothermia minus three degrees.

The animals were cooled using ice packs and warmed using a heat lamp. Each temperature-latency slope is based on 10 to 15 points (Figures 3 a. & 3 b.).

** Phase II - The Effect of Soman (GD) on ABR Latency:

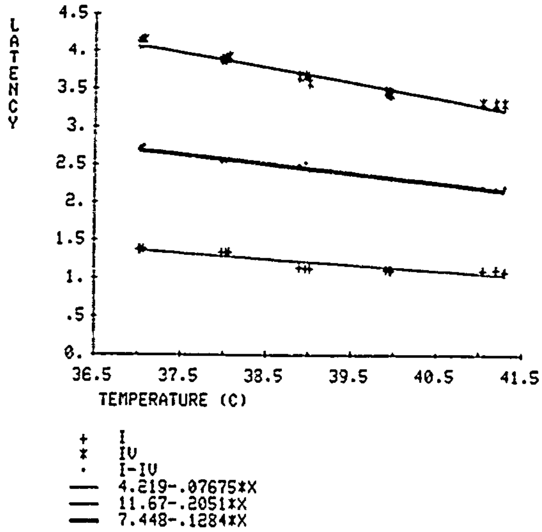
t = -60" baseline recordings
t = -30" atropine methyl nitrate (16 mg/kg, i.m.)
t = 00 soman (1 LD-50, s.c.)
t = +05 - 120" post soman recordings

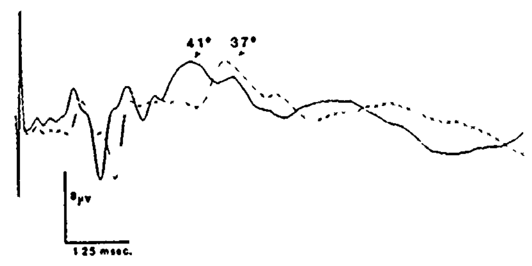
V - DATA ANALYSIS

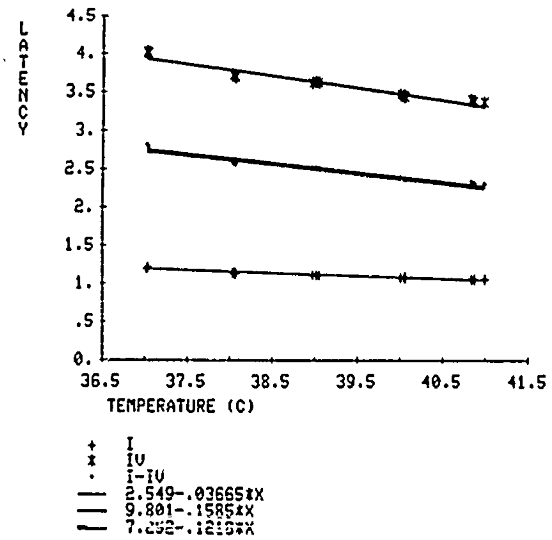
** the latency (in msec.) of the first peak (Wave I), the fourth peak (Wave IV) and the Inter-Peak Latency (IPL), the time between Wave I and IV, was determined for each waveform.

PHASE I

THE EFFECTS OF TEMPERATURE ON THE AUDITORY BRAINSTEM RESPONSE







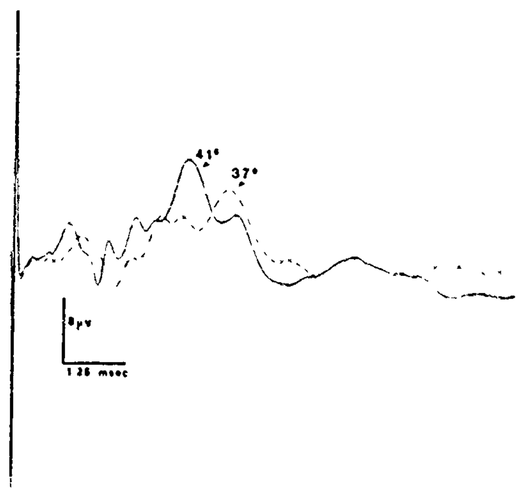


Figure 3-a.: LEFT: Graph of ABR latency vs. body temperature in two individual guinea pigs. Note that as temperature increases, ABR latency decreases. The slope of the regression line is steeper for the later components indicating a cumulative effect on latency.

Figure 3-b.: RIGHT: Corresponding waveforms recorded at maximum *(solid line)* and minimum *(dashed line)* temperatures during the temperature-latency study.

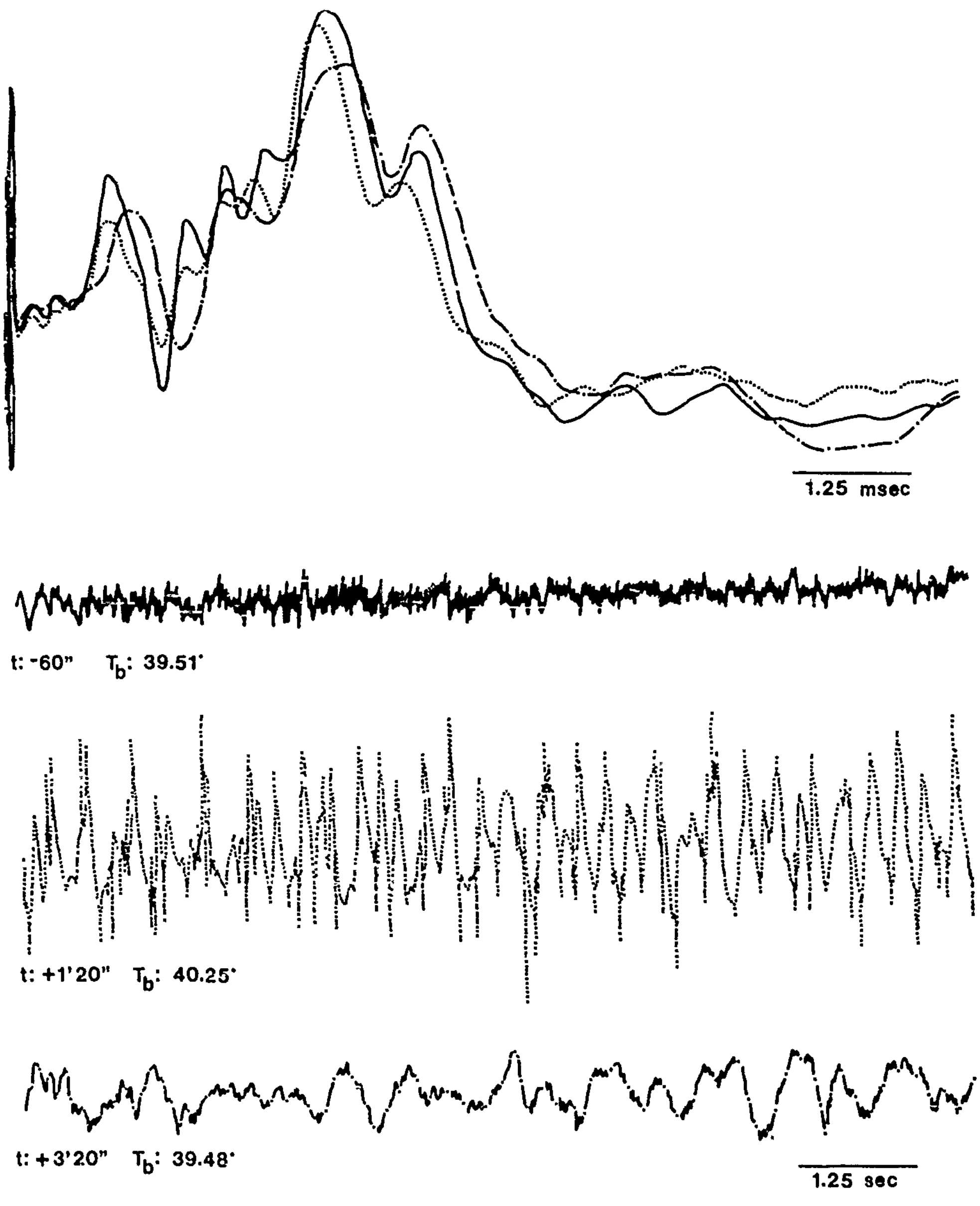


Figure 4.: ABR and EEG recorded during control *(red)*, during soman-induced seizure activity *(blue)*, and after seizure episode *(green)*. At the time of seizure *(blue)*, the body temperature was elevated relative to control, and the ABR latencies were decreased. After the seizure *(green)*, when body temperature return to control level, the latencies were increased.

PHASE II

THE EFFECTS OF SOMAN ON THE AUDITORY BRAINSTEM RESPONSE

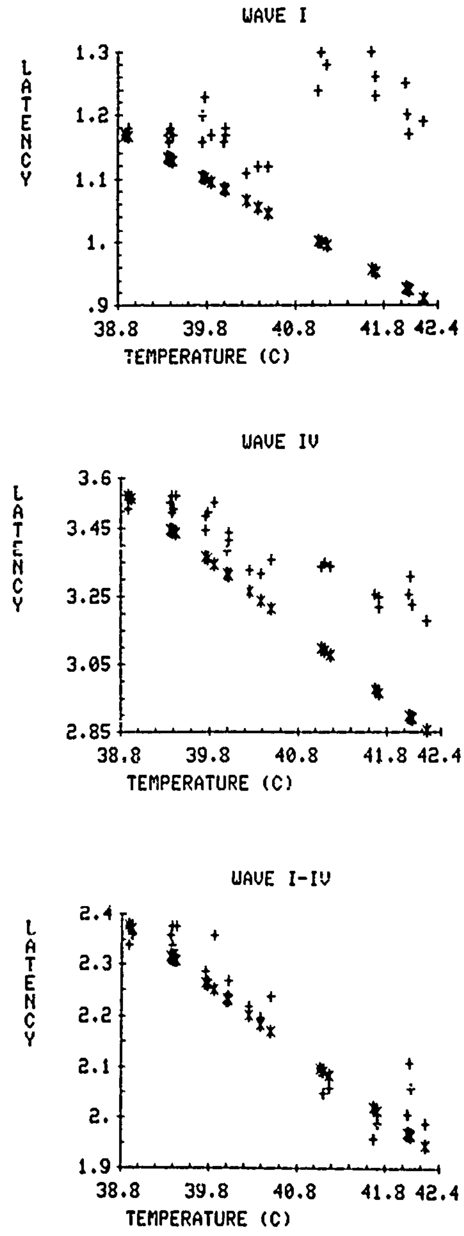


Figure 5.: Graph of body temperature vs. ABR latency for Observed (+) and "Predicted" (*) latencies of Wave I (left), Wave IV (middle) and I-IV IPL (right). Note that even after correction for changes in body temperature, soman significantly increased ABR latencies.

RESULTS

1.) The waveshape of the ABR changed in five out of six animals following administration of soman.

2.) Four of the animals developed a sustained seizure pattern following the soman. The onset of seizures typically occurred 30 minutes post-soman.

3.) Body temperature increased in two animals (range: 0.6 to 2.7 C) and decreased in four animals (range: 0.4 to 1.1 C) following soman administration. Room temperature was maintained within +/- 0.25 F.

4.) Several *within* animal comparisons were conducted on the latency of Waves I, IV and the I-IV IPL:

(a) Comparison of pre- versus post-soman latencies revealed that latencies significantly increased following administration of one LD 50 of soman (Mann-Whitney U Rank Sum Test) (see Table 1.).

(b) A "predicted" latency was computed for each animal using the temperature-latency regression formula determined during Phase I. Comparison of the observed post-soman latencies versus the "predicted" (temperature corrected) latencies revealed an effect of soman on the absolute latency of the ABR independent of temperature (paired t-test) in five of the six animals. Furthermore, neural conduction time (I-IV IPL) was significantly increased in three of the six animals.

TABLE 1: Effects of soman on electrophysiologic, physiologic and behavioral parameters: *within animal comparisons.*

			ABR Latencies					
No.	Temp.[1]	Seizure	Observed: Pre vs Post GD[1,3]			Post GD: Observed vs "Predicted"[2,4]		
			I	IV	IPL	I	IV	IPL
41	↑ **	0	*	*	*	**	**	**
42	↓ *	+	*(-)	*(-)	*(-)	**(-)	**(-)	**(-)
45	↓ **	0	*	**	**	*	**	**
50	↑ *	+	**	NS	NS	**	**	NS
52	↓ **	+	**	**	**	**	**	**
54	↓ **	+	**	**	*	**	**	NS

↑ *increased*
↓ *decreased*
* *p < .05*
** *p < .01*
0 *absent*
\+ *present*
1 *Mann Whitney U Rank Sum Test*
2 *paired t-test*
3 *In all cases, experimental latency was longer than control except where noted by (-).*
4 *In all cases, observed latency was longer than predicted except where noted by (-).*

DISCUSSION

1.) Soman changes the shape and latencies of the ABR. Both temperature dependent and temperature independent effects of soman on the ABR were observed.

2.) Unexpectedly, some animals became hyperthermic following the soman, not hypothermic.

3.) In some animals the threshold intensity for eliciting the ABR was elevated following soman.

4.) These data extend the findings of Bhargava et al. (1978) that cholinergic compounds adversely affect parameters of the Auditory Brainstem Response.

CONCLUSIONS

At least part of soman's effects can be attributed to changes in the animal's body temperature. A direct effect of soman on the ABR, independent of temperature, was also observed.

These preliminary data suggest that body temperature must be rigorously monitored and factored out in analyses to disassociate direct and indirect effects of nerve agent compounds on sensory processing.

ACKNOWLEDGEMENTS:

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SENIOR TECHNICIAN

CHOLINERGIC MECHANISMS AND INTERACTIONS IN MAMMALIAN RETINA: CYTOCHEMICAL LOCALIZATIONS

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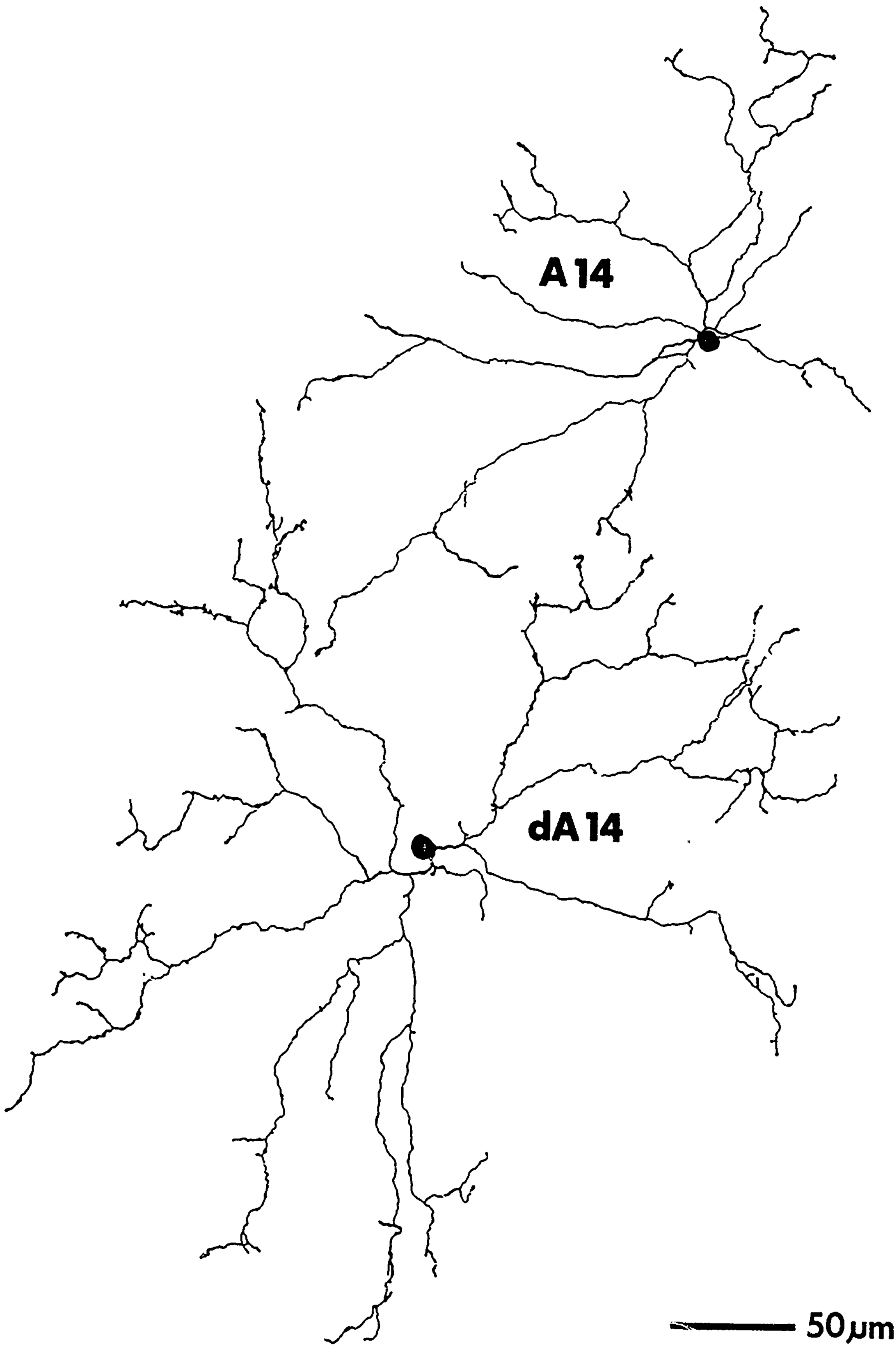
INTRODUCTION

THE CAT RETINA CONTAINS MORE THAN SIXTY MORPHOLOGICALLY DISTINCT SUBPOPULATIONS OF NEURONS. CYTOCHEMICAL STUDIES HAVE IDENTIFIED PUTATIVE NEUROTRANSMITTERS FOR A NUMBER OF THESE POPULATIONS. ALTHOUGH THERE IS SUBSTANTIAL PHYSIOLOGICAL EVIDENCE TO SUPPORT THE ROLE OF ACETYLCHOLINE (ACh) AS A NEUROTRANSMITTER IN CAT RETINA, THE SPECIFIC CELLS WHICH UTILIZE ACh HAVE NOT BEEN PREVIOUSLY IDENTIFIED. THE GOAL OF THESE CYTOCHEMICAL STUDIES HAS BEEN TO IDENTIFY THE CHOLINERGIC CELLS IN THE CAT RETINA AND TO DETERMINE HOW THESE CELLS COMPARE WITH THOSE CELLS WHICH CONTAIN THE HYDROLYZING ENZYME ACETYLCHOLINESTERASE (AChE). THESE STUDIES WILL SERVE AS THE BASIS FOR INVESTIGATION OF THE INTERACTIONS OF ACh AND AChE WITHIN THE CAT RETINA AND THE EFFECTS OF ANTI-CHOLINESTERASES ON RETINAL TRANSMITTER SYSTEMS.

STARBURST-LIKE AMACRINE CELLS



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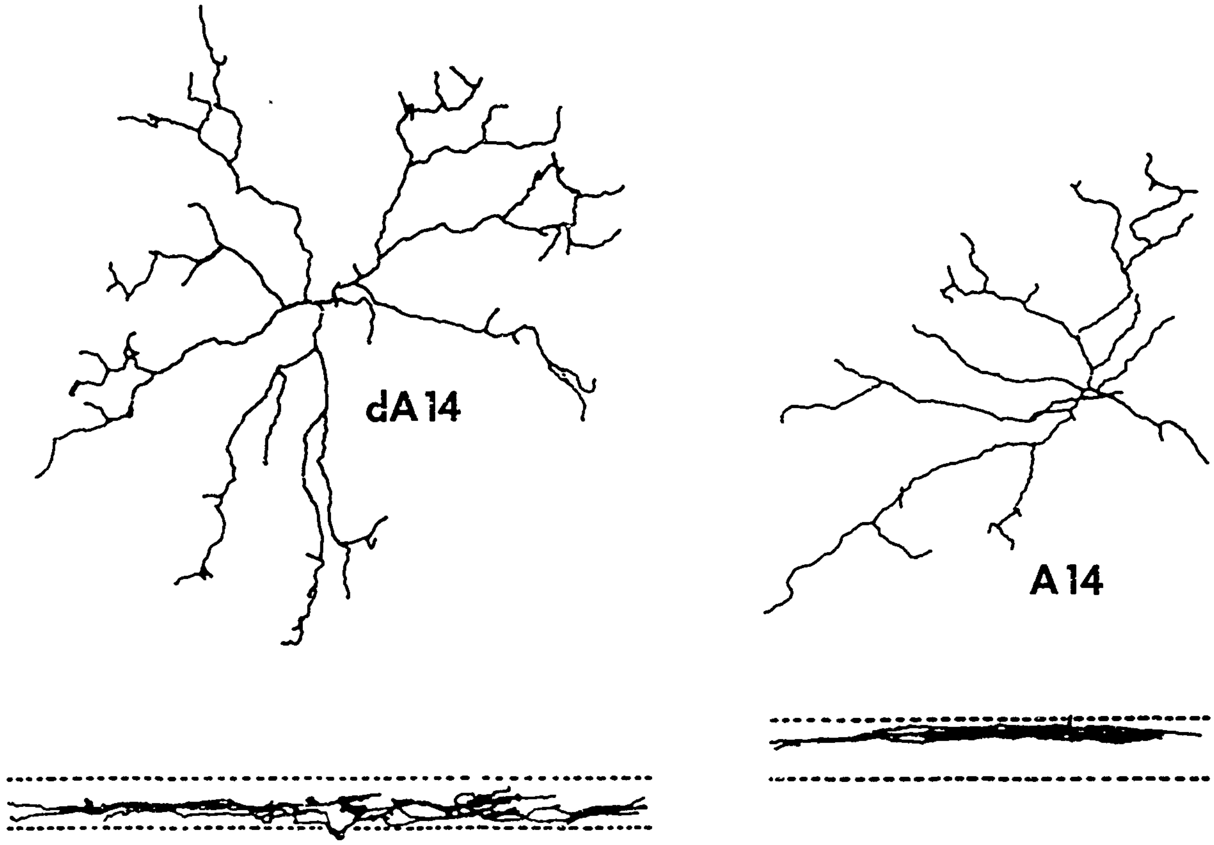


CHOLINERGIC CELLS HAVE BEEN WELL DEFINED IN THE RABBIT RETINA WHERE THEY COMPRISE MATCHING SUBPOPULATIONS OF AMACRINE AND DISPLACED AMACRINE CELLS WITH A MIRROR-SYMMETRIC DISTRIBUTION ON EITHER SIDE OF THE INNER PLEXIFORM LAYER (IPL) AND PROCESSES RAMIFYING IN STRATA 2 AND 4 OF THE IPL (MASLAND AND MILLS, 1979). IN GOLGI PREPARATIONS, THESE CELLS EXHIBIT A CHARACTERISTIC STARBURST APPEARANCE WITH A DICHOTOMOUS BRANCHING PATTERN (FAMIGLIETTI, 1983). OUR GOLGI STUDIES HAVE REVEALED THE PRESENCE OF SIMILAR CELLS IN THE CAT RETINA, ALTHOUGH THE DENDRITES BRANCH MORE SPARSELY THAN IN THE RABBIT. THE DISPLACED CELLS APPEAR IDENTICAL TO THE A14 AMACRINES DESCRIBED BY KOLB ET AL. (1981) AND HAVE BEEN DESIGNATED dA14 WHILE THE AMACRINE LAYER CELLS WERE DESIGNATED A14.

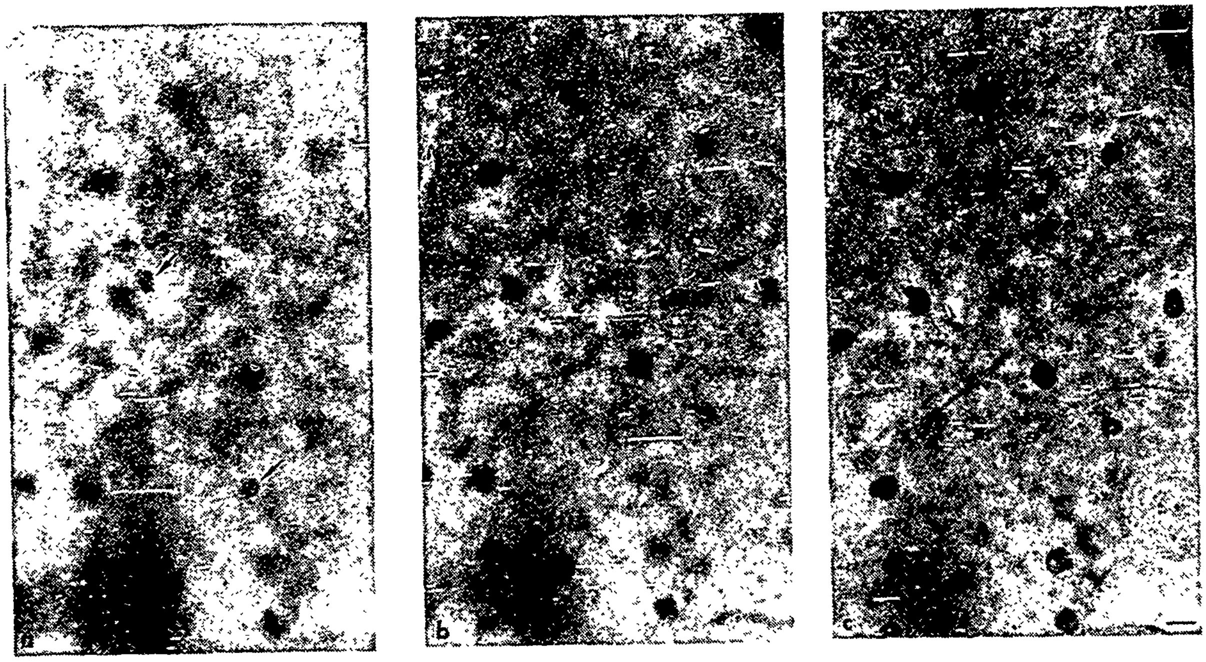
BOTH TYPES OF STARBURST-LIKE AMACRINE CELL WERE LOGGED FOR COMPUTER RECONSTRUCTION AND ROTATED AROUND THE X-AXIS. A14 AMACRINES WERE FOUND TO RAMIFY IN STRATUM 2 OF THE INNER PLEXIFORM LAYER WHILE dA14 CELLS WERE SEEN TO RAMIFY IN STRATUM 4.

SYNTHESIS OF ACETYLCHOLINE

IN ORDER TO DETERMINE WHETHER THE STARBURST-LIKE CELLS ARE CHOLINERGIC, CAT RETINAS WERE INCUBATED WITH 0.3 uM (3H)CHOLINE FOLLOWED BY A CHASE IN UNLABELED CHOLINE CONTAINING 20 nM Mg AND 30 uM PHYSOSTIGMINE. THESE CONDITIONS AS DESCRIBED BY MASLAND AND MILLS (1979) ARE DESIGNED TO RETAIN NEWLY SYNTHESIZED (3H)ACh. TISSUE WAS THEN FIXED IN 3% PHOSPHOMOLYBDIC ACID (TSUJI, 1984) AND 2% GLUTARALDEHYDE AND PROCESSED FOR AUTORADIOGRAPHY. LABELING WAS SEEN IN BOTH AMACRINE AND DISPLACED AMACRINE CELLS (ARROWS). SOME ACCUMULATION OF LABEL WAS ALSO OBSERVED IN PHOTORECEPTOR OUTER SEGMENTS AND IN LARGE GANGLION CELLS. LABELING OF AMACRINE CELLS IS CONSISTENT WITH THE POSSIBILITY THAT THESE NEURONS ARE SYNTHESIZING (3H)ACh WHILE RADIOACTIVITY IN PHOTORECEPTOR AND GANGLION CELLS MAY REFLECT HIGH LEVELS OF (3H)CHOLINE INCORPORATION INTO PHOSPHOLIPIDS.

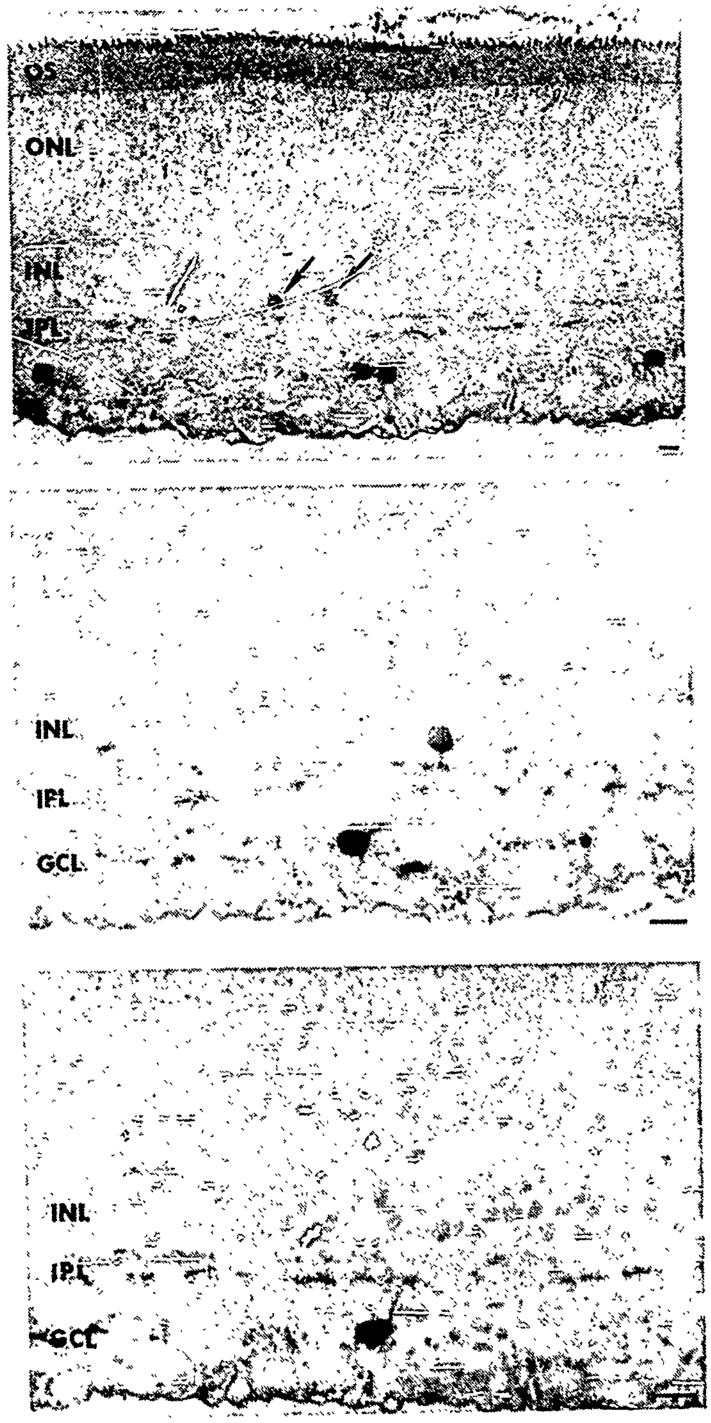
OS, OUTER SEGMENTS; ONL, OUTER NUCLEAR LAYER; OPL, OUTER PLEXIFORM LAYER; INL, INNER NUCLEAR LAYER; IPL, INNER PLEXIFORM LAYER; GCL, GANGLION CELL LAYER; BAR, 10 um.

CHOLINE ACETYLTRANSFERASE

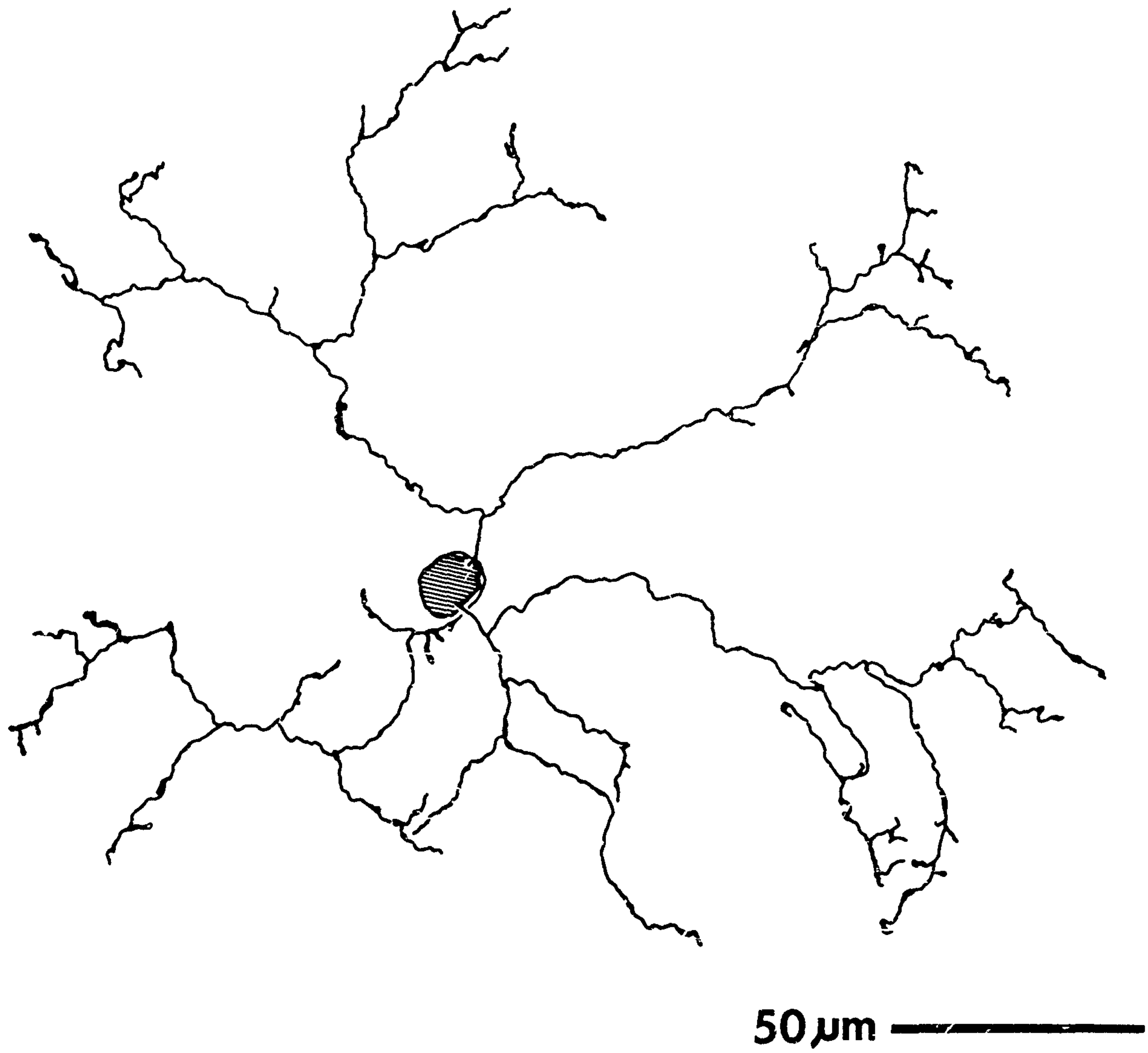


THE MOST WIDELY ACCEPTED TECHNIQUE FOR CYTOCHEMICAL IDENTIFICATION OF CHOLINERGIC NEURONS IS THE IMMUNOCYTOCHEMICAL LOCALIZATION OF THE SYNTHESIZING ENZYME, CHOLINE ACETYLTRANSFERASE (ChAT). CAT RETINAS WERE FIXED FOR 30 MIN IN 4% PARAFORMALDEHYDE IN 0.1 M PHOSPHATE BUFFER, THEN SUNK IN 30% SUCROSE. TISSUE WAS TREATED WITH 0.3% TRITON X-100 AND REACTED OVERNIGHT WITH A RAT MONOCLONAL ANTISERUM (4 mg/ml) AGAINST PORCINE ChAT OBTAINED FROM

BOEHRINGER-MANNHEIM. IMMUNOREACTIVITY WAS VISUALIZED BY SEQUENTIAL REACTIONS WITH RABBIT ANTI-RAT IgG (1:20), RAT PEROXIDASE ANTIPEROXIDASE (1:100), AND DIAMINOBENZIDINE. FLAT-MOUNT PREPARATIONS OF CAT RETINA SHOWED ChAT IMMUNOREACTIVITY IN (a) AMACRINE CELLS, (b) PROCESSES RAMIFYING WITHIN THE INNER PLEXIFORM LAYER, AND (c) DISPLACED AMACRINE CELLS. THE NUMBER OF REACTIVE SOMAS IN THE GANGLION CELL LAYER OUTNUMBERED THE NUMBER IN THE AMACRINE LAYER BY APPROXIMATELY 3:1.



IN RADIAL SECTIONS, ChAT IMMUNOREACTIVITY WAS SEEN IN AMACRINE CELLS (ARROWS) WITH SOMAS IN THE INNER NUCLEAR LAYER (INL) AND IN DISPLACED AMACRINE CELLS WITH SOMAS IN THE GANGLION CELL LAYER (GCL). REACTIVE PROCESSES FORM NARROW BANDS IN THE INNER PLEXIFORM LAYER (IPL) AT APPROXIMATELY S2 AND S4. ChAT REACTIVE AMACRINE CELLS SEND PRIMARY DENDRITES INTO S2 OF THE IPL WHILE DISPLACED AMACRINE CELLS CONTAINING ChAT RAMIFY IN S4.

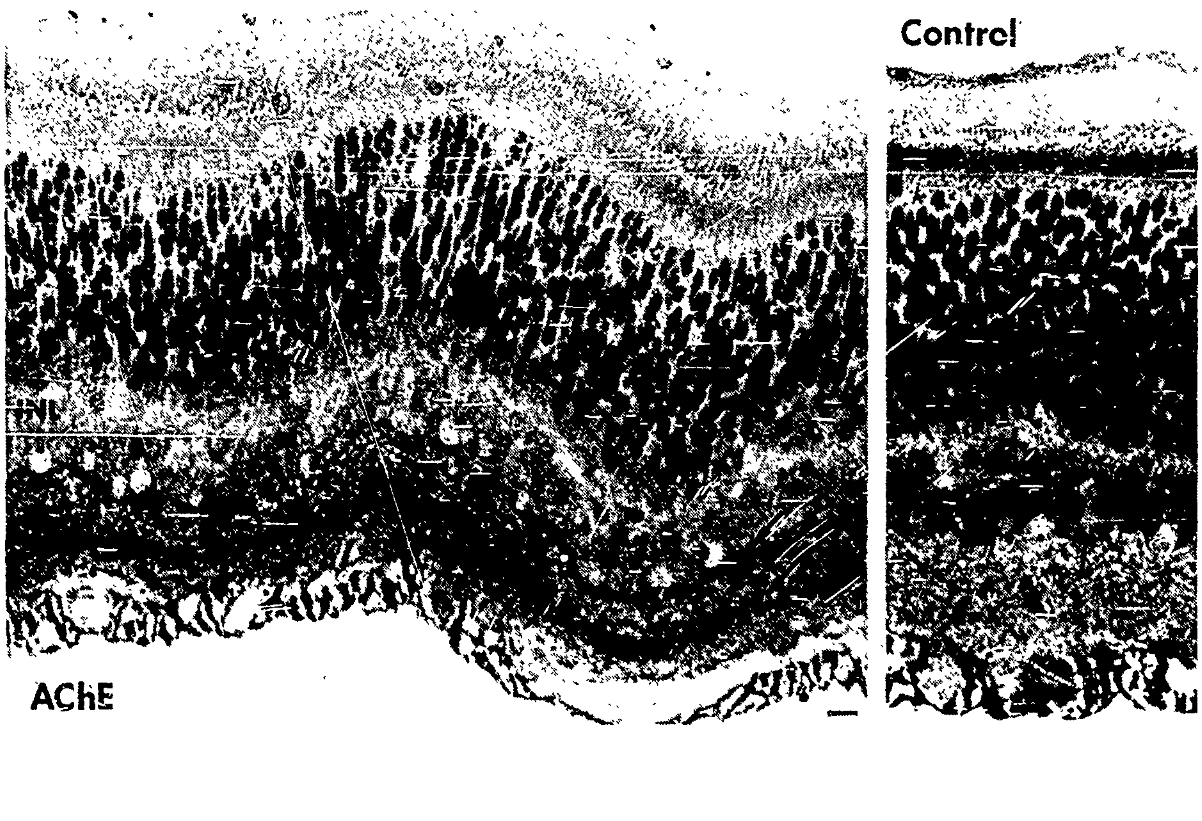


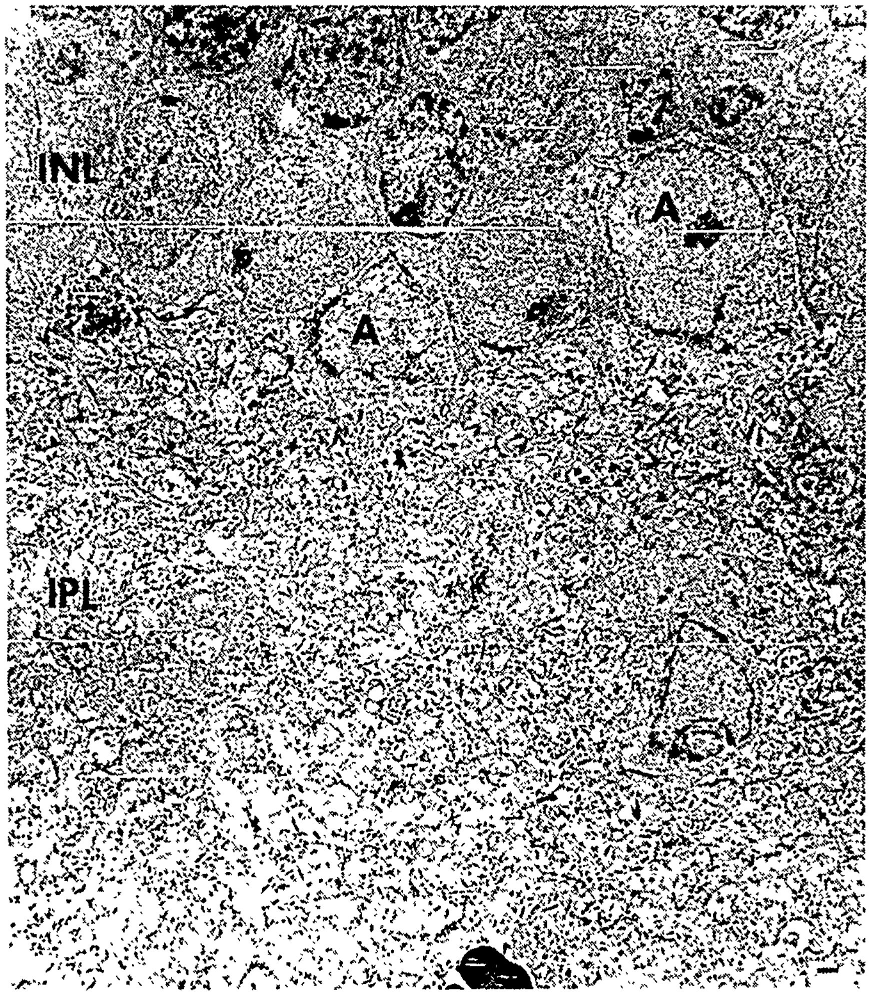
A CAMERA LUCIDA DRAWING OF A ChAT IMMUNOREACTIVE DISPLACED AMACRINE CELL SHOWS THE SAME MORPHOLOGICAL FEATURES AS THE A14 AND dA14 CELLS OBSERVED IN GOLGI STUDIES. THIS CONFIRMS THE IDENTIFICATION OF THESE AMACRINE CELLS AS CHOLINERGIC.

A-1059

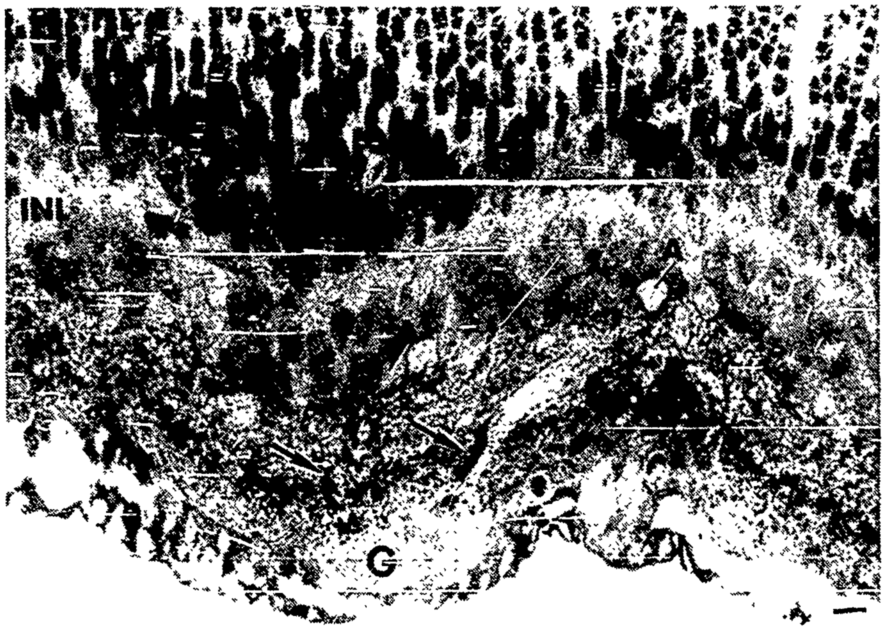
ACETYLCHOLINESTERASE

THE ACh HYDROLYZING ENZYME, AChE, WAS LOCALIZED IN CAT RETINA BY A MODIFICATION OF THE KOELLE-FRIEDENWALD TECHNIQUE DESCRIBED BY VAN OOTEGHEM AND SHIPLEY (1984). RETINAS WERE LIGHTLY FIXED IN 4% PARAFORMALDEHYDE AND 0.2% GLUTARALDEHYDE AND THE SUBSTRATE USED WAS ACETYLTHIOCHOLINE IODIDE. REACTION PRODUCT WAS CONFINED TO THE INNER PLEXIFORM LAYER AND TO CELL BODIES OF NEURONS BORDERING THIS LAYER. BANDING WITHIN THE IPL APPEARS AT THE LEVEL OF STRATA 1 AND 4. A CONTROL RETINA INCUBATED IN THE PRESENCE OF 10^{-5} M BW 284 TO INHIBIT SPECIFIC AChE ACTIVITY SHOWED NO REACTION PRODUCT, INDICATING THAT ESSENTIALLY ALL OF THE CHOLINESTERASE ACTIVITY IN THE CAT RETINA IS SPECIFIC FOR ACh.

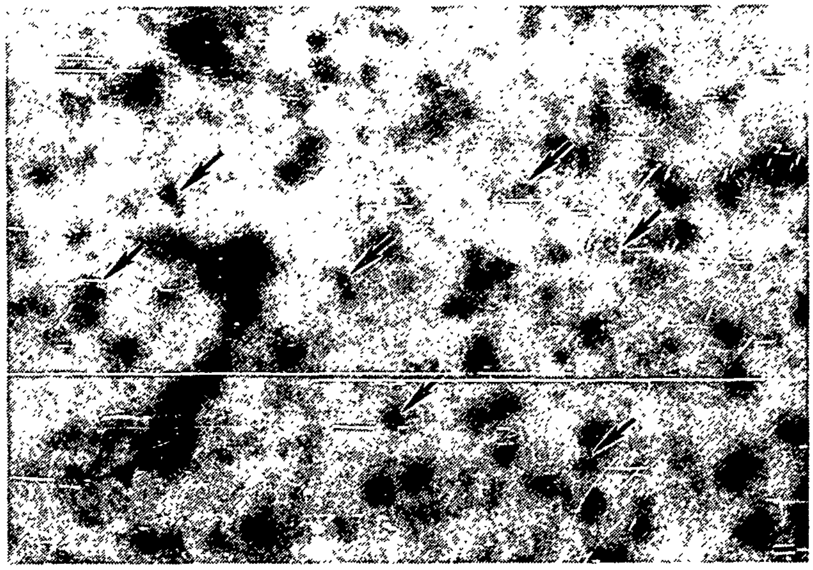


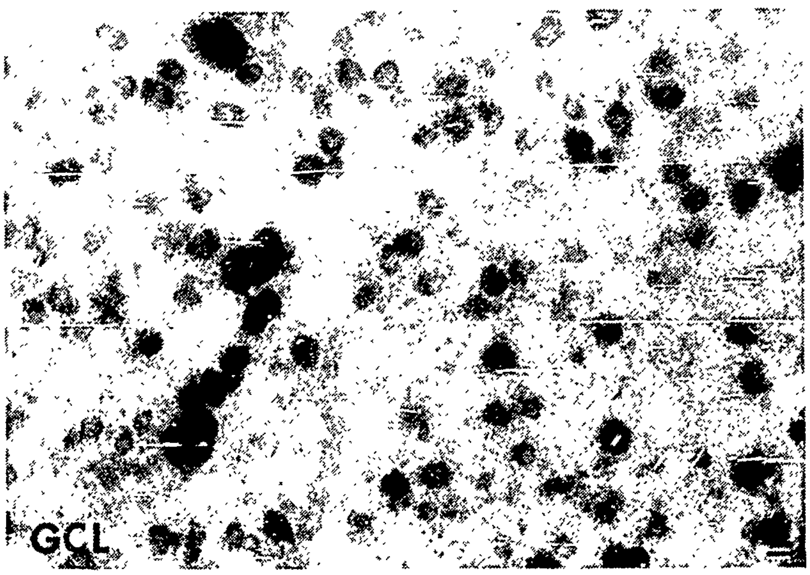


AT THE ELECTRON MICROSCOPE LEVEL, AChE REACTION PRODUCT WAS SEEN THROUGHOUT THE INNER PLEXIFORM LAYER AND IN AT LEAST TWO TYPES OF AMACRINE CELL. THE MORE STRONGLY REACTIVE CELL WAS A SMALL NEURON WITH A SOMA APPROXIMATELY 8 um IN DIAMETER WHILE THE OTHER MEASURED 12 um IN DIAMETER.

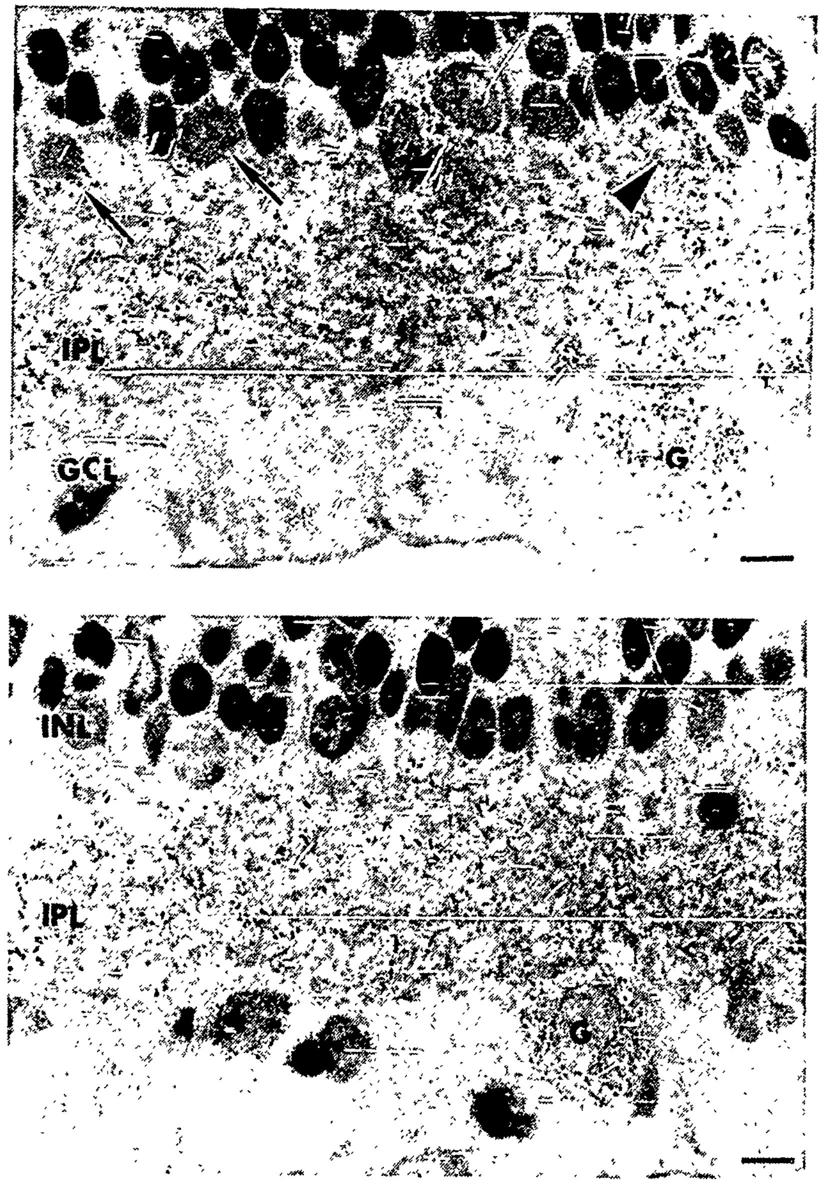


AT HIGHER MAGNIFICATION, AChE REACTION PRODUCT WAS SEEN IN CELL BODIES OF SOME AMACRINE CELLS AND IN MANY CELLS IN THE GANGLION CELL LAYER. DENDRITES OF ALPHA TYPE GANGLION CELLS SHOWED ESPECIALLY LARGE ACCUMULATIONS (ARROW).





SOME CATS WERE PRETREATED WITH DIISOPROPYLFLUOROPHOSPHATE (DFP) 6-10 HRS PRIOR TO SACRIFICE AND THE RETINAS WERE PROCESSED FOR AChE REACTIVITY. FLAT MOUNT PREPARATIONS SHOWED NEWLY SYNTHESIZED AChE IN A SMALL NUMBER OF AMACRINE CELLS AND IN ESSENTIALLY ALL OF THE GANGLION LAYER CELLS.



RADIAL SECTIONS OF THESE RETINAS CONFIRMED THE PRESENCE OF AChE IN BOTH SMALL (SMALL ARROWS) AND LARGE (LARGER ARROWS) AMACRINE CELLS. GANGLION LAYER CELLS WERE CONSISTENTLY REACTIVE.

AN ELECTRON MICROGRAPH OF A GANGLION CELL FROM A DFP TREATED CAT SHOWS THE PRODUCTION OF NEW AChE WITHIN THE NUCLEAR ENVELOPE AND BY PROFILES OF ROUGH ENDOPLASMIC RETICULUM.

CONCLUSIONS

1. CAT RETINA CONTAINS AMACRINE AND DISPLACED AMACRINE CELLS WHICH ARE MORPHOLOGICALLY SIMILAR TO CHOLINERGIC CELLS IN OTHER MAMMALIAN RETINAS.

2. THESE STARBURST-LIKE CELLS RAMIFY IN STRATA 2 AND 4 OF THE INNER PLEXIFORM LAYER AS DO CHOLINERGIC CELLS OF RABBIT, RAT, PIG, CHICKEN, AND GOLDFISH.

3. CELLS IN THE POSITION OF AMACRINE AND DISPLACED AMACRINE CELLS APPEAR ABLE TO SYNTHESIZE ACETYLCHOLINE FROM (3H)CHOLINE.

4. CELLS IDENTIFIABLE AS STARBURST-LIKE AMACRINE CELLS (A14 and dA14) ARE IMMUNO-REACTIVE FOR THE ACETYLCHOLINE SYNTHESIZING ENZYME, CHOLINE ACETYLTRANSFERASE.

5. THE ACETYLCHOLINE HYDROLYZING ENZYME, ACETYLCHOLINESTERASE, IS LOCALIZED NOT ONLY IN THE CHOLINERGIC CELLS BUT ALSO IN OTHER AMACRINE CELLS AND ESSENTIALLY ALL OF THE GANGLION CELLS. THE ROLE OF AChE IN THESE CELLS REMAINS TO BE DETERMINED.

PHARMACODYNAMICS OF THE PUPIL AND ACCOMMODATION RESPONSE TO INTRAMUSCULAR ATROPINE IN HUMANS

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US Army Medical Research Institute of Chemical Defense
Aberdeen Proving Ground, Maryland 21010-5425

PROCEDURE

- DEVELOP PHARMACOKINETIC MODEL
- DEVELOP RESPONSE MODEL
- CALCULATE EFFECTS
- COMPARE TO PUBLISHED OBSERVATIONS

A mathematical model was developed to predict the effect of intramuscular (I.M.) atropine on pupil diameter and accommodative range in the human eye. Atropine is a primary constituent of the US Armed Forces' antidotes for nerve gas. Following I.M. injection, atropine paralyzes the iris sphincter and the ciliary muscle. As a result, pupil diameter is increased and accommodation range is decreased. Using published plasma concentrations following intravenous (I.V.) and I.M. injection, drug concentrations in the tissue were estimated with a two-compartment first order model with zero order absorption. Pupil and accommodation changes were predicted using a Michaelis-Menton model based on published responses from the in vitro mouse iris. The results of the pharmacokinetic (distribution) analysis of the published plasma concentration data provided rate constants with which tissue concentrations were predicted. Calculated pupil and accommodation changes were compared to the published measurements for.5, 1, 2, and 4 mg, I.M. doses. For both the pupil diameter and residual accommodation, the time course of the predicted responses was faster than that of the averaged published measurements. The difference between predicted and published maximum responses for pupil width change was 7%. Excluding the 4 mg dose of atropine, predicted accommodative changes were about half as large as published responses. The results support the use of several assumptions inherent in the model. First, the predicted tissue concentrations are reasonable estimates for the iris. Second, the mouse in vitro data can be used to represent the human response. And last, the accuracy of the model for pupil response, unlike that for accommodation, is independent of dose. In summary, a simple two compartment model can be used to estimate maximum pupil response from I.M. atropine. Predictions of an accurate time course of response, and of the accommodative response in general, require a more complex model.

RESPONSE PARAMETERS

$$\Omega = \frac{R}{R_{max}-R}$$

Ω = RESPONSE PARAMETER
R = RESPONSE

FOR EXAMPLE:

$$\Omega\ \text{PUPIL} = \frac{D-D_o}{D_{max}-D}$$

$$\Omega\ \text{ACCOMMODATION} = \frac{\Delta_o-\Delta}{\Delta}$$

$$\text{Log}\ \Omega = \text{M Log C} + \text{B}$$

D = PUPIL DIAMETER
D_{max} = MAXIMUM PUPIL DIAMETER
D_o = PUPIL DIAMETER (TIME = 0)
Δ = RESIDUAL ACCOMMODATION
Δ_o = RESIDUAL ACCOMMODATION (TIME = 0)

Fig. 1 PROCEDURE SCHEMATIC

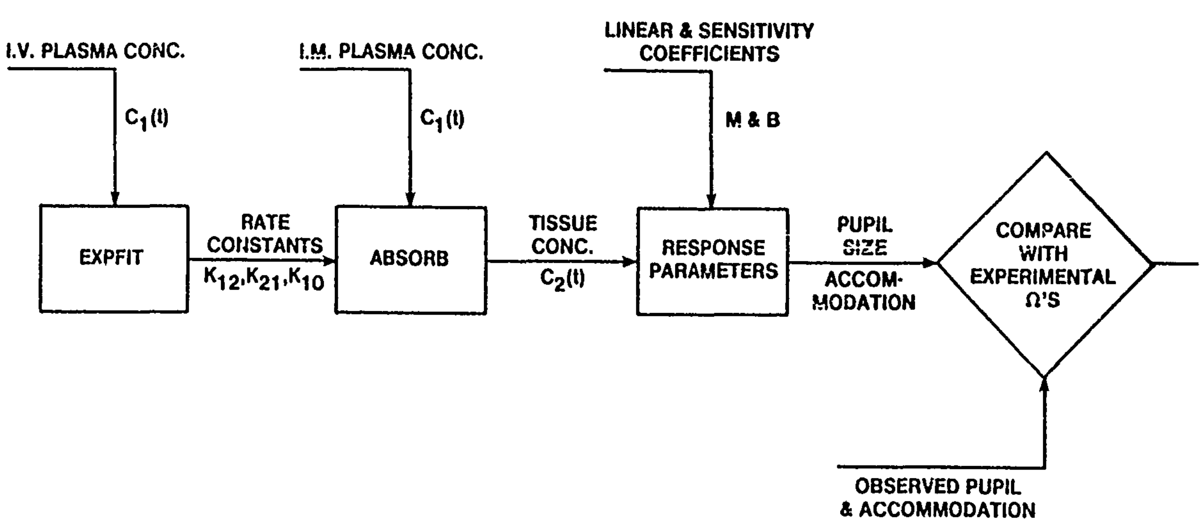


Fig. 2 TWO-COMPARTMENT PHARMACOKINETIC MODEL

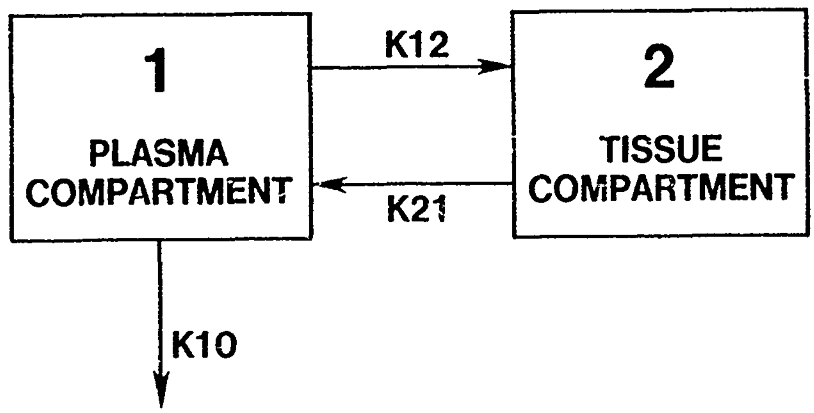


Fig. 3 CURVE FIT TO PLASMA CONCENTRATIONS OF BERGHEM, et al., (1980) (1 mg DOSE) FOR $C_1(t) = 96\ EXP(-.77\ t) + 15\ EXP(-.042\ t)$

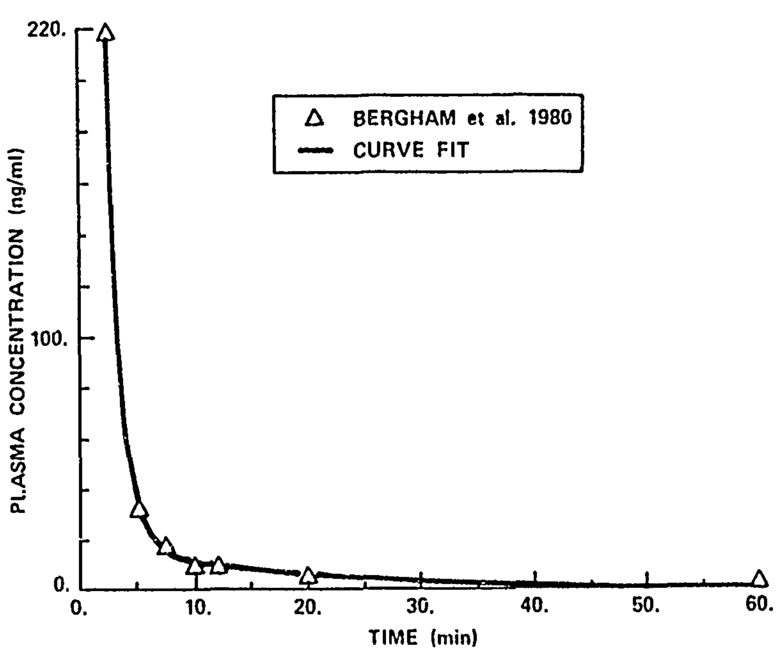


Fig. 4 CURVE FIT TO PREDICTED TISSUE CONCENTRATION NORMALIZED TO 1 mg DOSE $C_2(t) = 11\ EXP(-.0047\ t) - 12\ EXP(-.031\ t)$

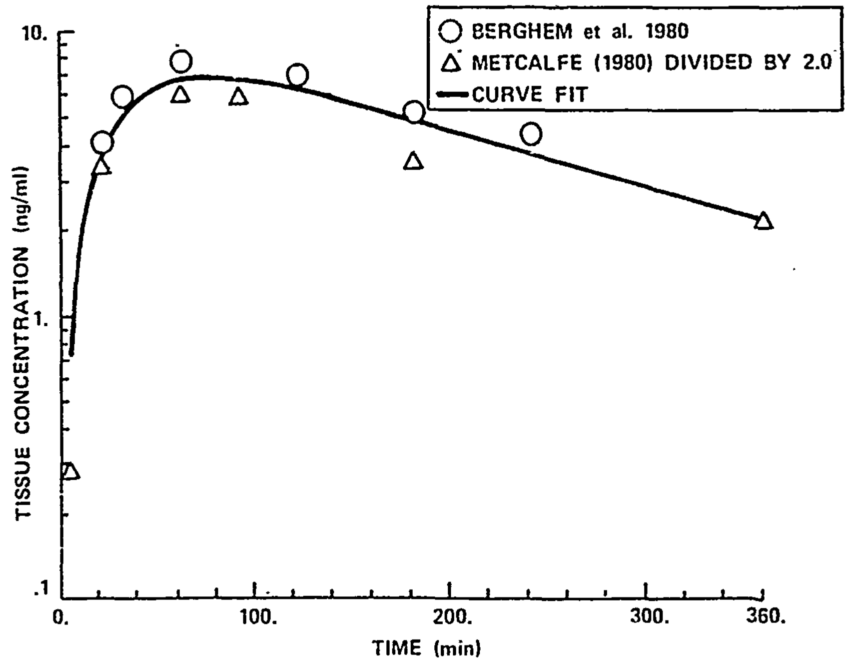


Fig. 5 LINEAR REGRESSION TO MOUSE IN VITRO DATA (BEAVER & RIKER, 1962)

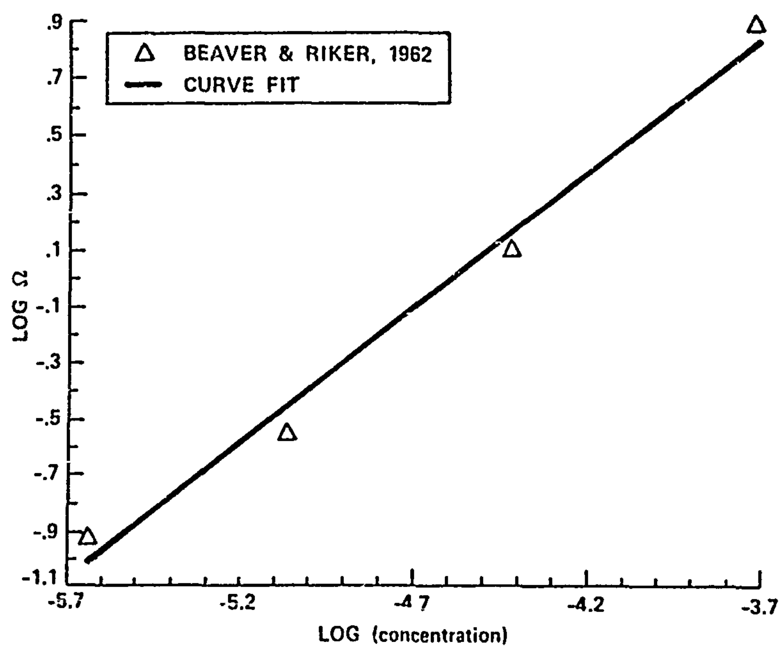


Fig. 7 PUPIL RESPONSE FOR A 1 mg I.M. INJECTION

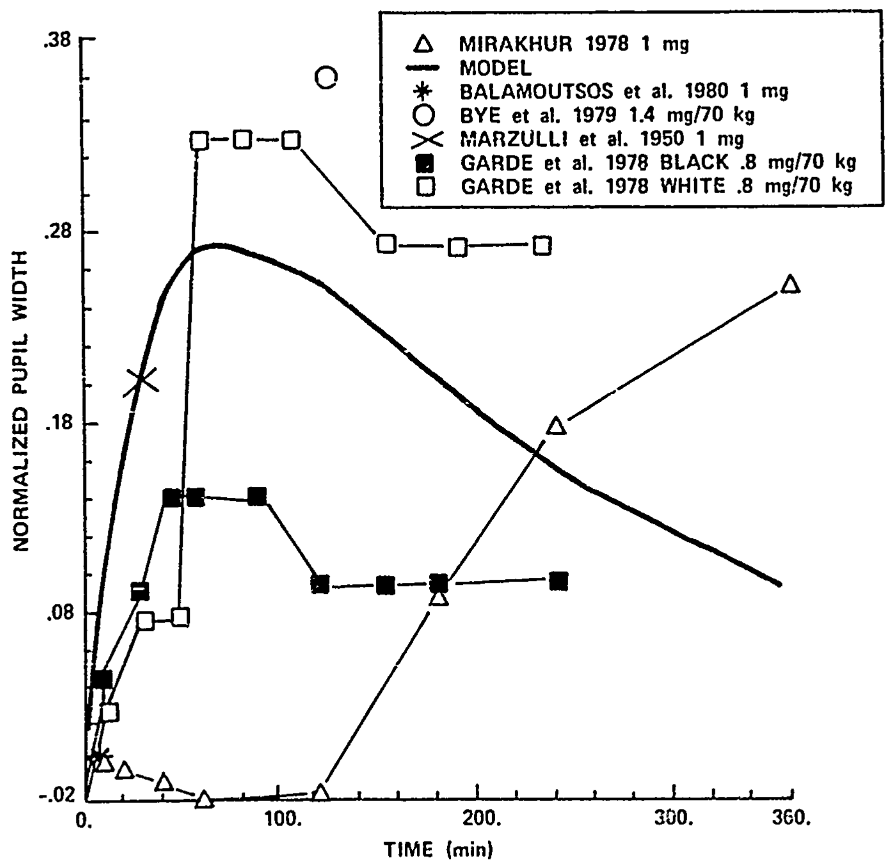


Fig. 8 PUPIL RESPONSE FOR A 2 mg I.M. INJECTION

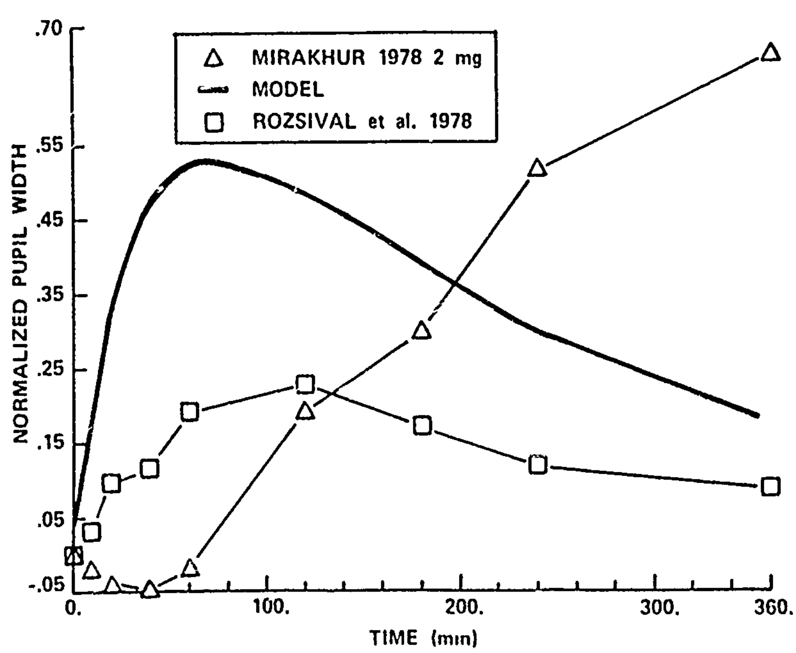


Fig. 9 PUPIL RESPONSE FOR A 4 mg I.M. INJECTION

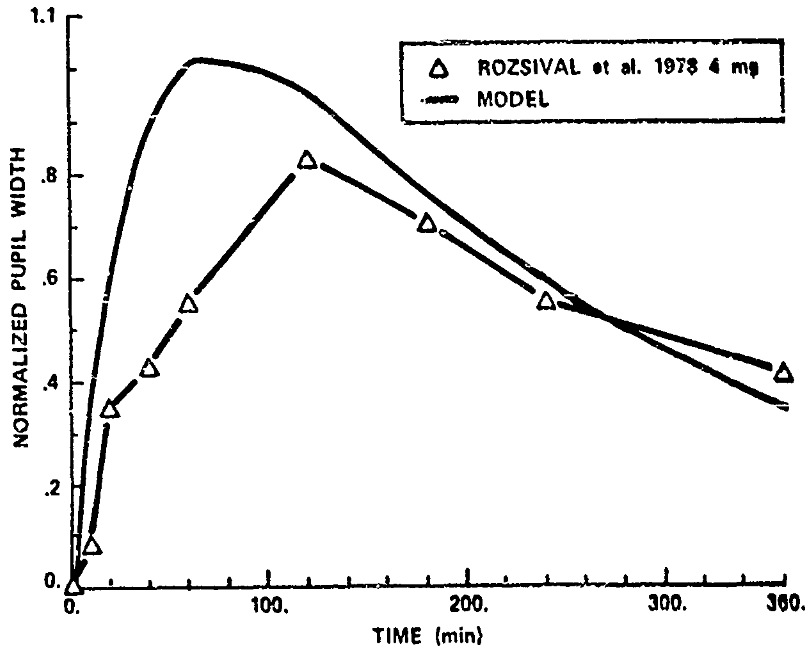


Fig. 11 ACCOMMODATIVE RESPONSE FOR A 1 mg I.M. INJECTION

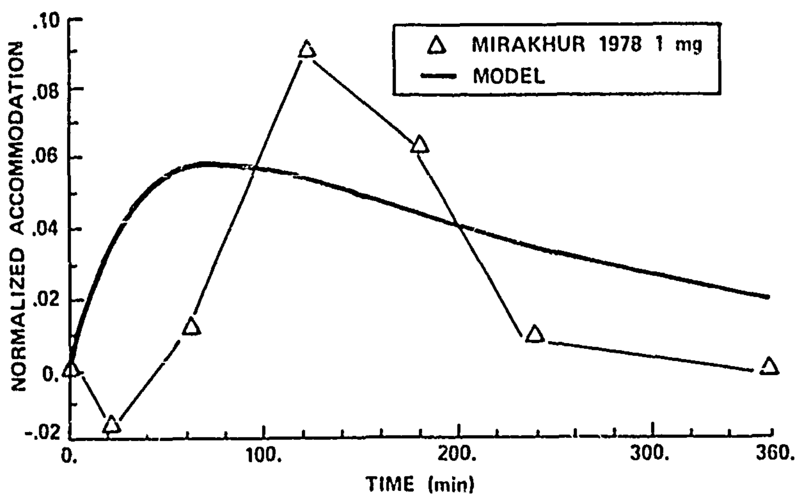


Fig. 12 ACCOMMODATIVE RESPONSE FOR A 2 mg I.M. INJECTION

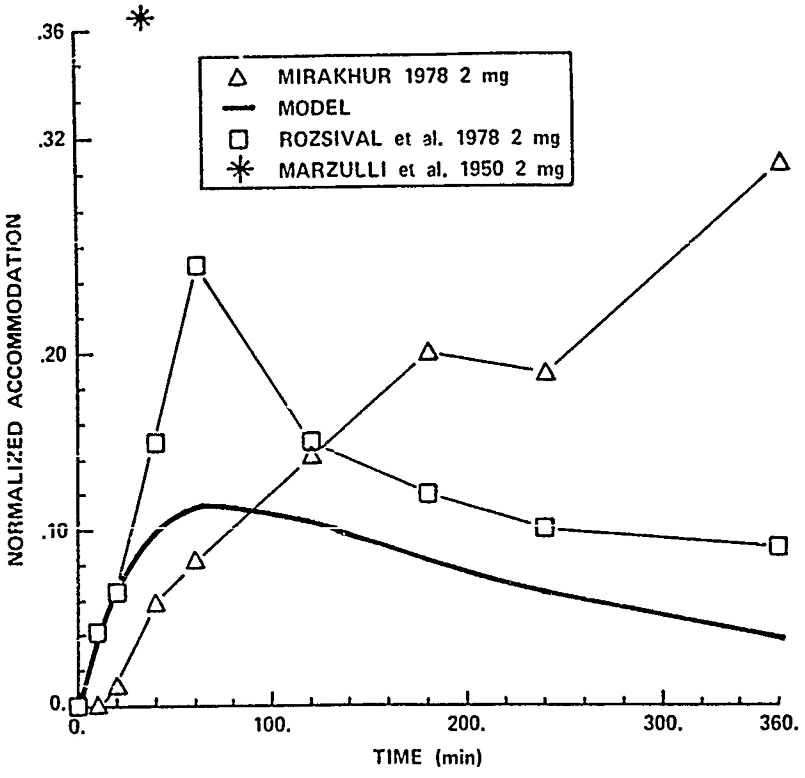
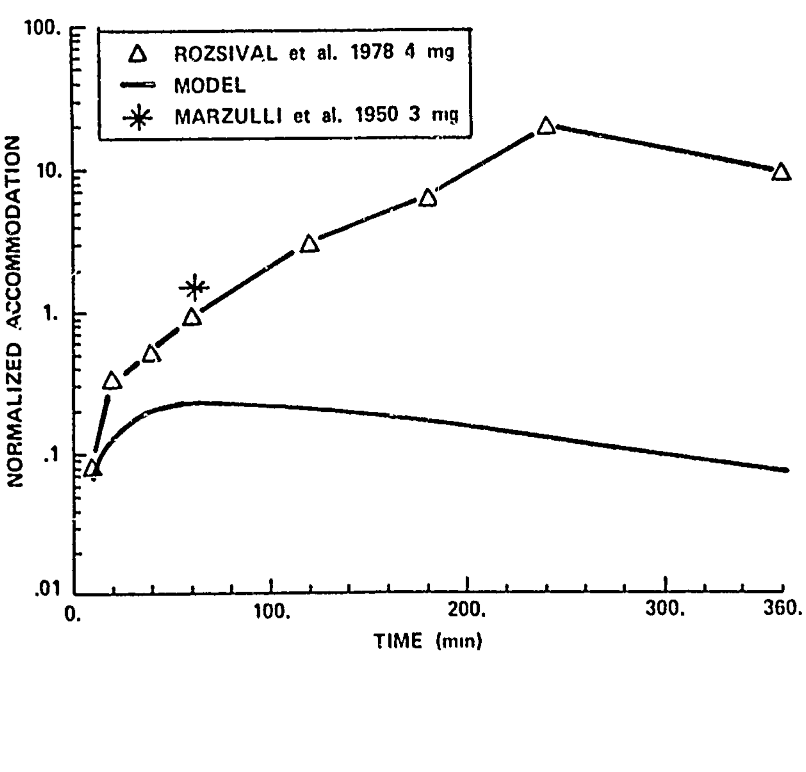
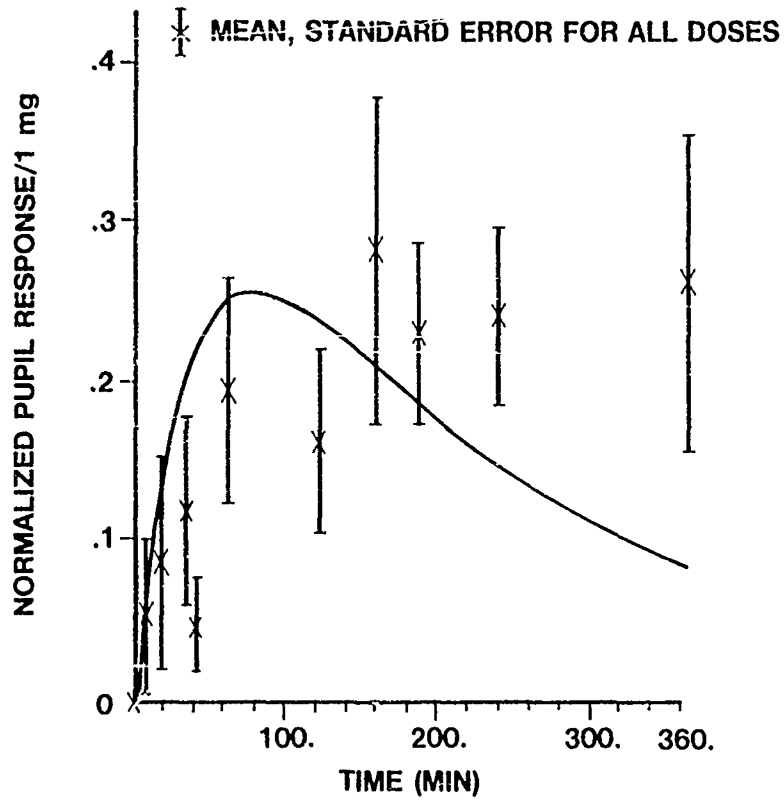


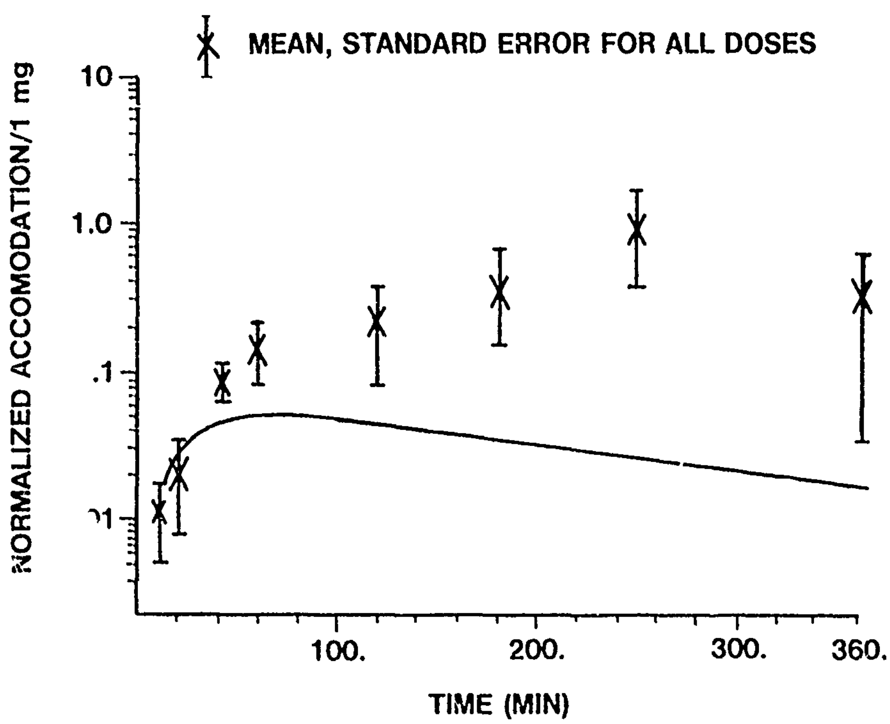
Fig. 13 ACCOMMODATIVE RESPONSE FOR A 4 mg I.M. INJECTION



PUPIL RESPONSE FOR ALL DOSES



ACCOMMODATIVE RESPONSE FOR ALL DOSES



CONCLUSIONS

- DATA NEEDED AT HIGH DOSES
- MODEL PREDICTS MAXIMUM PUPIL RESPONSE
- MODEL IS TOO SIMPLE TO PREDICT MAXIMUM ACCOMMODATION OR TIME COURSE

10. Neurobiological Correlates

ADVERSE CNS EFFECTS OF ATROPINE AFTER CHOLINESTERASE INHIBITION BY DFP, SOMAN AND SARIN

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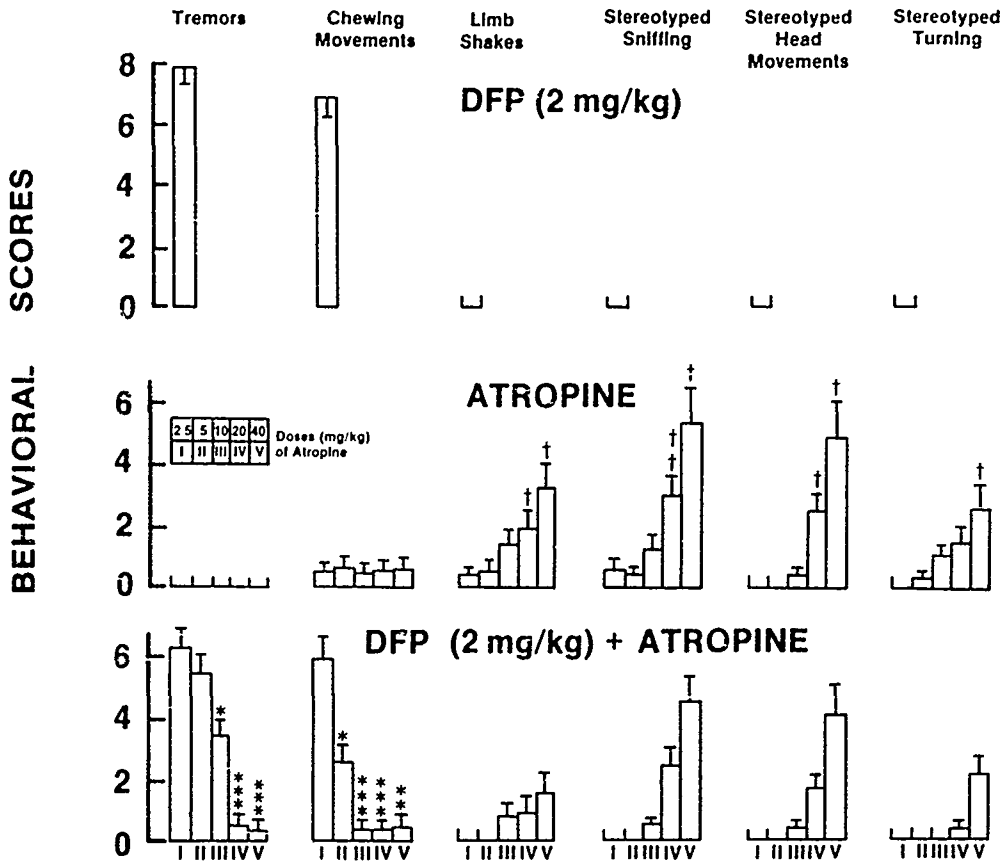
BEHAVIOURAL EVALUATION *

SCORE	TREMORS	OTHER BEHAVIOURAL RESPONSES
0	NOT DISPLAYED	NOT DISPLAYED
1	SLOW TREMOR OF HEAD	SLIGHT
2	FAST TREMOR OF HEAD, TRUNK OR LIMBS	MODERATE
3	INTENSE " " " " "	SEVERE
(4	EXTREMELY INTENSE " " " " "	EXTREMELY SEVERE)

FOR 5 MINUTE PERIODS, AT 15 MIN. INTERVALS, FOR EACH BEHAVIOUR, ON EACH ANIMAL, AND 1-HOURLY SUMMED SCORES USED.

* FERNANDO ET AL, 1984, EUR. J. PHARMACOL. ; 1984, PHARMACOL. BIOCHEM. BEHAV.
FERNANDO AND CURZON, 1981, NEUROPHARMACOL.

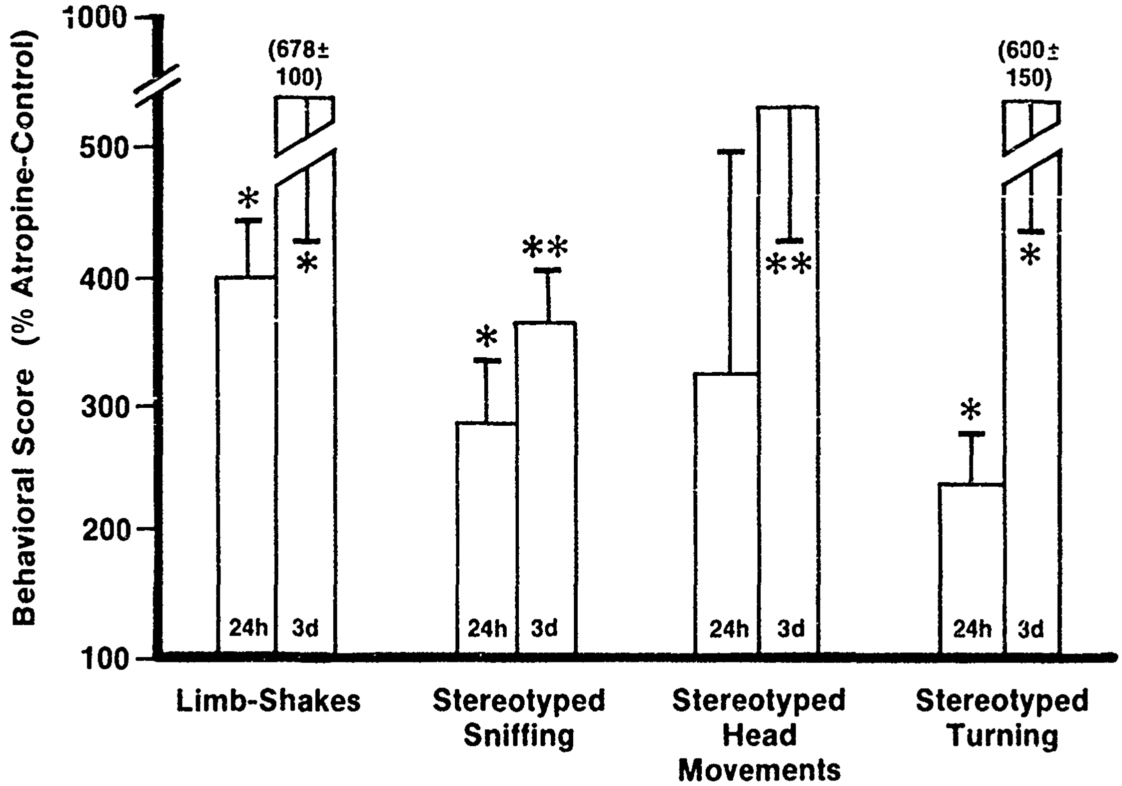


DFP PRODUCED TREMORS AND CHEWING MOVEMENTS MAXIMALLY DURING THE SECOND HOUR AFTER INJECTION AND THEY DISAPPEARED AFTER ABOUT SIX HOURS IN RATS.

ATROPINE TREATMENT ALONE ELICITED A BEHAVIORAL SYNDROME CONSISTING OF INTERMITTENT RAPID RHYTHMIC LIMB SHAKES, OCCASIONAL BODY SHAKES AND EPISODES OF THE STEREOTYPED ACTIVITIES, NAMELY, REPETITIVE SNIFFING, FAST UP AND DOWN MOVEMENTS OF THE HEAD AND COMPULSIVE TURNING OR WALKING. ONLY LIMB SHAKES AND SNIFFING WERE DISPLAYED TO A SLIGHT DEGREE AFTER GIVING 2.5 AND 5.0 MG/KG OF ATROPINE. HOWEVER, AFTER 20 MG/KG ATROPINE, HEAD MOVEMENTS AND TURNING ALSO OCCURRED. ALL OF THESE EFFECTS GOT MORE INTENSE AND FREQUENT WITH INCREASING DOSAGES UP TO ABOUT 40 MG/KG, BEYOND WHICH NO FURTHER EFFECTS WERE OBSERVED.

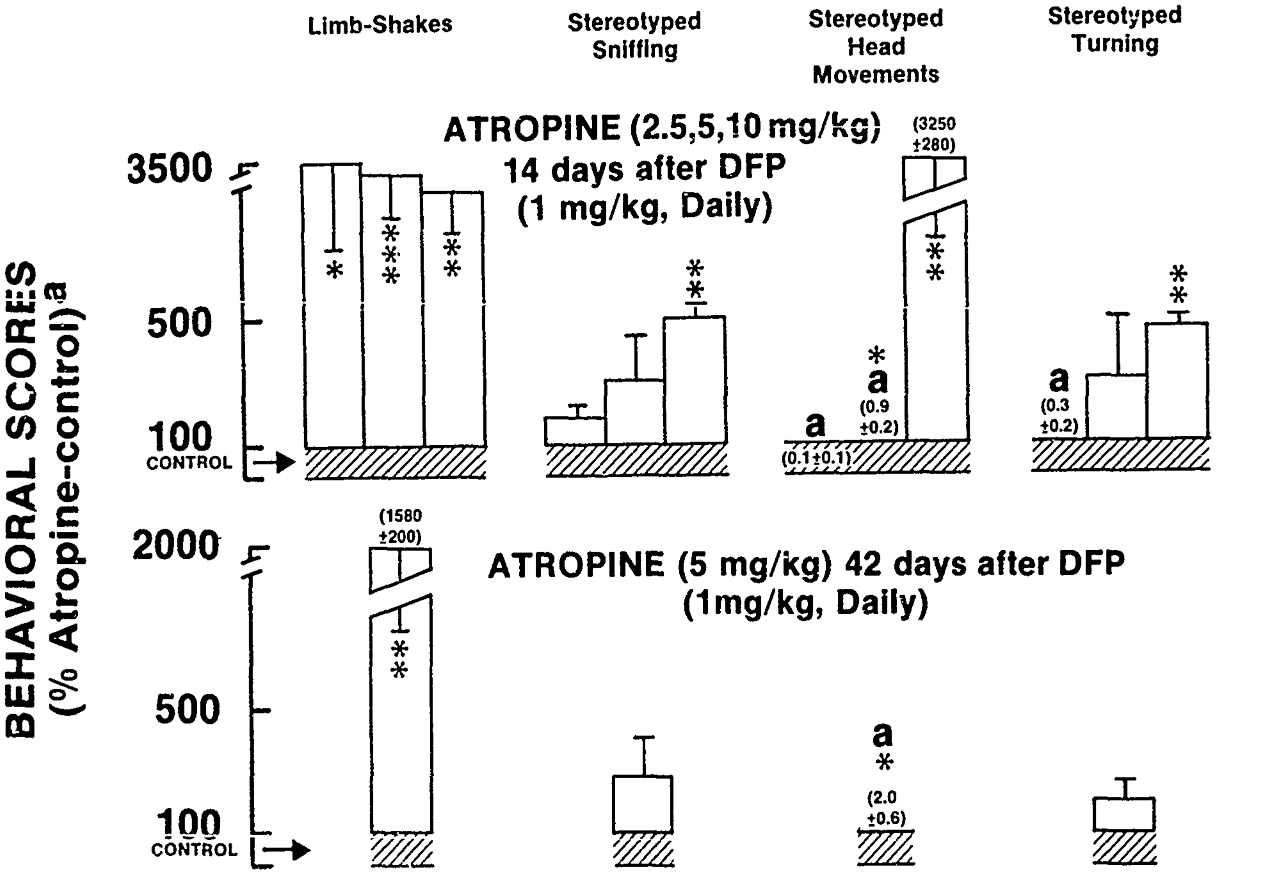
THE ANTAGONISM OF DFP-INDUCED BEHAVIORS BY ATROPINE WAS STUDIED ONE HOUR AFTER INJECTING DFP. ADMINISTRATION OF ATROPINE SULFATE (2.5 - 40 MG/KG) BLOCKED THE TREMORS AND CHEWING MOVEMENTS DOSE-DEPENDENTLY: 20 OR 40 MG/KG, ALMOST COMPLETELY; 5 AND 10 MG/KG, PARTLY; WHILE 2.5 MG/KG HAD NO EFFECT.

ATROPINE-INDUCED (10mg/Kg) RESPONSES AFTER SINGLE TREATMENT WITH DFP (2 mg/Kg)



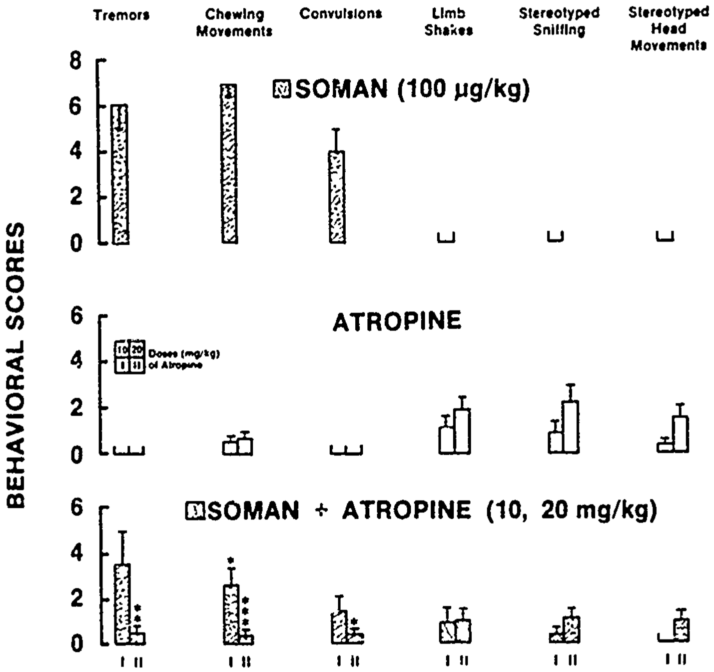
TWENTY-FOUR HOURS AFTER DFP, SOME OF THE BEHAVIORS, PARTICULARLY THE LIMB SHAKES, INDUCED BY ATROPINE AT DOSES OF 5 AND 10 MG/KG WERE SIGNIFICANTLY INCREASED.

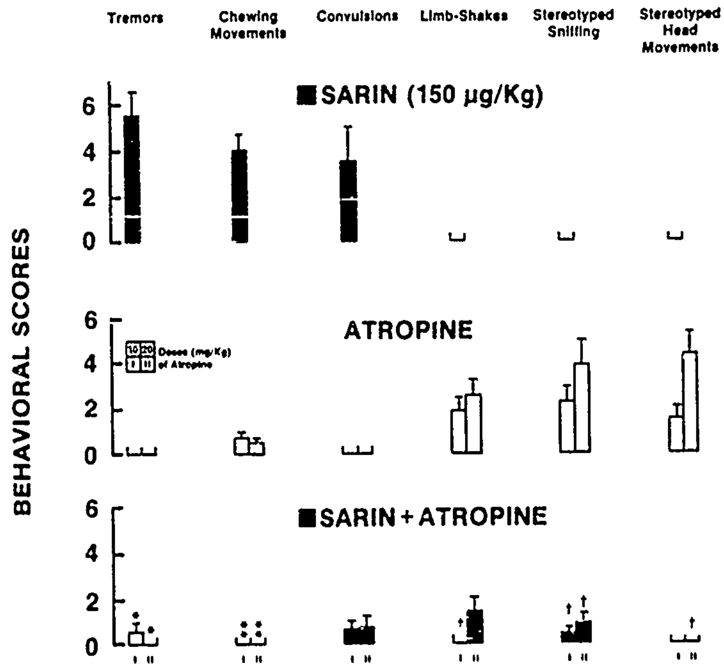
THREE DAYS AFTER DFP, ALL FOUR RESPONSES INDUCED BY ATROPINE AT DOSES OF 10 AND 20 MG/KG WERE SIGNIFICANTLY ENHANCED.



IN ANIMALS RECEIVING 14 DAILY INJECTIONS OF DFP, 1 MG/KG, SIGNIFICANT ENHANCEMENT OF ATROPINE-INDUCED BEHAVIORS, LIMB SHAKES IN PARTICULAR, WAS EVIDENT EVEN AT THE DOSE OF 2.5 MG/KG.

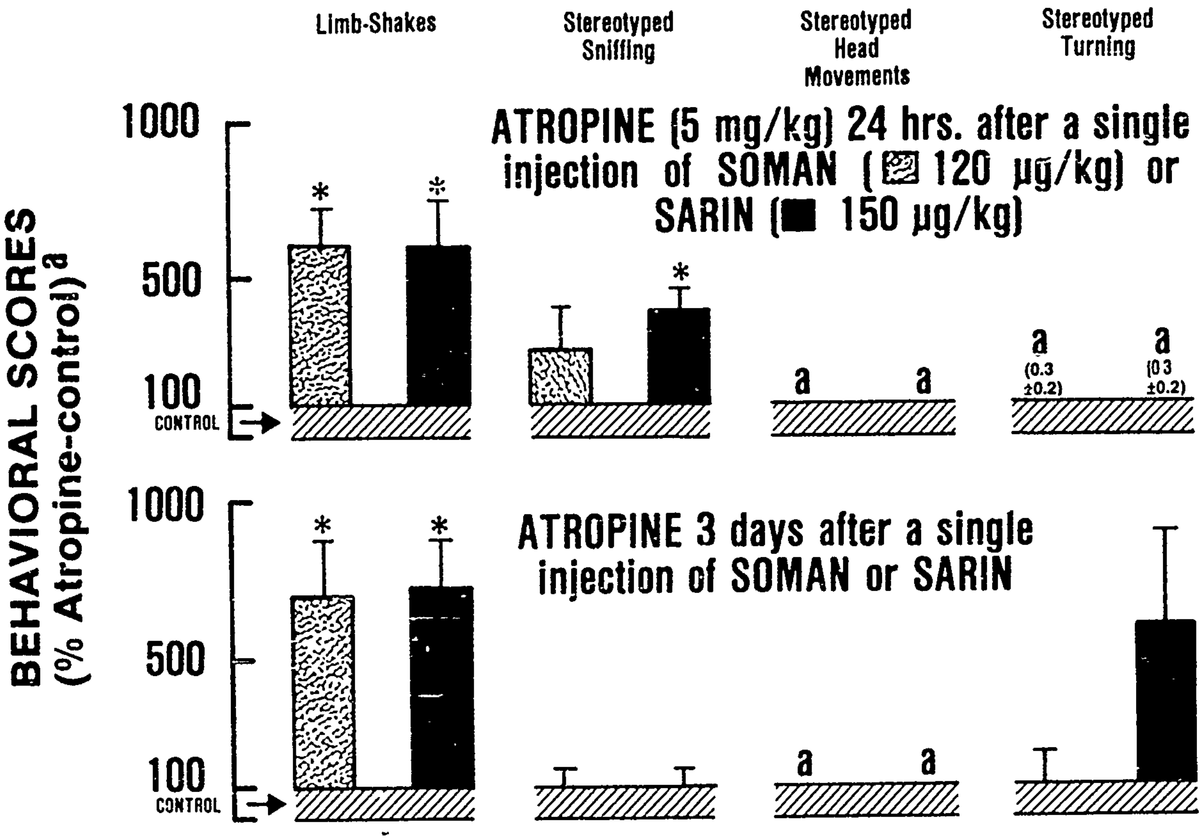
SIMILARLY, SIX WEEKS AFTER DFP, LIMB SHAKES AND HEAD MOVEMENTS CAUSED BY ATROPINE AT THE DOSE OF 5 MG/KG WERE GREATLY ENHANCED.





POTENT ORGANOPHOSPHATE CHOLINESTERASE INHIBITORS WERE ALSO STUDIED. SOMAN (100 μG/KG) AND SARIN (150 μG/KG) CAUSED TREMORS, CLONIC-TONIC CONVULSIONS AND CHEWING MOVEMENTS WITHIN 10 - 30 MIN AFTER INJECTION AND THEY LASTED FOR 2 - 6 HOURS, ALTHOUGH OCCASIONALLY CLONIC CONVULSIONS PERSISTED FOR ABOUT 24 HOURS.

ATROPINE AT DOSES OF 10 AND 20 MG/KG INJECTED 15 MINUTES BEFORE, ALMOST TOTALLY ABOLISHED THE SOMAN- AND SARIN-INDUCED EFFECTS. ON THE OTHER HAND, THE ATROPINE-INDUCED RESPONSES WERE ATTENUATED BY BOTH SOMAN AND SARIN.



TWENTY-FOUR HOURS AFTER TREATMENT WITH SOMAN, THE LIMB SHAKES PRODUCED BY ATROPINE WERE SIGNIFICANTLY INCREASED AND SARIN TREATMENT LED TO SIGNIFICANT ENHANCEMENT OF THE LIMB SHAKES AND SNIFFING.

THREE DAYS AFTER TREATMENT WITH SOMAN OR SARIN, ATROPINE-INDUCED LIMBSHAKES WERE SIGNIFICANTLY INCREASED. THE BASE-LINE STEREOTYPED BEHAVIORS OF THE SOMAN- AND SARIN-TREATED RATS WERE NOT QUALITATIVELY DIFFERENT FROM THOSE OF THE CONTROLS EXCEPT FOR VARYING DEGREES OF WEIGHT LOSS AND LETHARGY, PARTICULARLY AT 24 HOURS AFTER TREATMENT.

CONCLUSION

THESE STUDIES SUGGEST THAT DEPENDING ON THE TIME INTERVAL AND DURATION OF ADMINISTRATION OF THESE ORGANOPHOSPHATE CHOLINESTERASE INHIBITORS AND THE ADMINISTRATION OF ATROPINE, ATROPINE CAN INDUCE ADVERSE EFFECTS. (THESE STUDIES WERE SUPPORTED BY USAMRDC #DAMD17-85-C5036.)

LONG-TERM REDUCTIONS OF AChE IN CORTICAL CHOLINERGIC TARGET SITES AFTER A SINGLE SOMAN CHALLENGE

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ABSTRACT

Acetylcholinesterase (AChE) is present in cholinergic neurons--their cell bodies, dendrites, axons, and terminals--and probably in cholinoceptive target neurons. The enzyme is thus widely distributed throughout cholinergic circuits yet its critical functional site is at the cholinergic synapse where it inactivates acetylcholine by hydrolysis. A central issue in Soman toxicity, therefore, is the degree and rate of recovery of AChE at cholinergic synaptic sites. Most measures of AChE recovery in brain, however, are based on biochemical determinations in homogenates of brain tissue. In such measures the small contribution to total AChE activity made by the functionally relevant synaptic fraction is relatively small in comparison to the high levels of the enzyme present in the other elements of cholinergic circuitry (cell bodies, dendrites, axons). Thus there is currently little information available on the recovery of AChE at cholinergic synaptic sites in the brain.

The successful protection and treatment of military personnel exposed to sublethal challenges of Soman will probably hinge on our understanding of Soman's action at central cholinergic synaptic sites because recent evidence suggests that specific reductions of cholinergic markers in cerebral cortical structures is correlated with, and may be the cause of, the severe declines of memory, cognition and higher mental function in Alzheimer's disease.

We have begun to study the recovery of AChE in cholinergic synaptic target structures using both biochemical assays and image analysis based densitometric measures of AChE stained with a new

histochemical procedure (Van Ooteghem and Shipley, 1984). Our approach has been based on the use of a unique model cholinergic circuit--the cholinergic projection from the nucleus of the diagonal band (NDB) to the olfactory bulb (OB) in the rat. The key feature of this model is that NDB is the sole source of cholinergic input to OB; OB contains no intrinsic cholinergic neurons. Thus, the olfactory bulb is a purely cholinoceptive target site. To date we have studied animals exposed to a single challenge of 0.7LD50 Soman.

Biochemical assays of whole forebrain samples including the cerebral cortex indicate that AChE is recovering to normal levels by 16-20 days. However, in the olfactory bulb--a purely cholinoceptive structure--AChE is still depressed by 50% at 16 days. When brains from similarly treated animals are stained for AChE and measured by densitometry, it is clear that both the bulb and the cerebral cortex are still depressed at 16 days. Densitometric analysis of cerebral cortex and olfactory bulb from animals allowed to survive for longer times indicate that in some cases AChE is still markedly depressed after two months or longer.

These findings indicate that cortical synaptic target sites have a hitherto unsuspected, prolonged vulnerability to Soman. In view of mounting evidence that reductions of cortical cholinergic synaptic function underly the devastating losses of higher mental function and memory in victims of Alzheimer's disease our results indicate that additional study of Soman's action on cholinergic synapses in cortical targets is vitally needed.

This work was supported in part by US Army Medical Research and Development Command under contract DAMD 17-82-C-2272 and by DOD DAAG 29-83-C-0064.

HYPOTHESIS

AChE's major role is to regulate ACh at synapses. Biochemical data (right) suggest cortical AChE recovers more slowly than sites containing cholinergi neurons. Thus we hypothesize that cortical cholinergic synapses may be particularly vulnerable to soman's actions.

MODEL CORTICAL CHOLINERGIC SYSTEMS

CORTICAL CHOLINERGIC MODEL

Our basic model system is a cholinergic projection from the nucleus of the diagonal band (NDB) to the olfactory bulb. This block documents the neuroanatomical features which make the NDB->OB circuit ideally suited to the study of a forebrain->cortical cholinergic circuit.

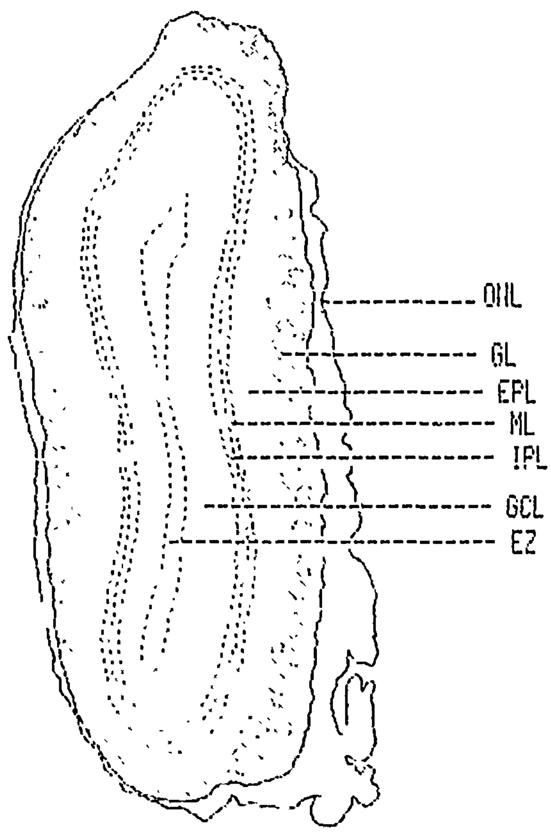
(i.) NDB is the only source of cholinergic input to OB--there are no intrinsic cholinergic neurons in OB. Thus, OB is a purely cholinoceptive cortical target.

(ii.) Neurotoxin lesions (ibotenic acid) of DB eliminate all AChE staining in OB.

(iii.) Fibers from DB terminate in specific layers of OB. These same layers are rich in AChE and (not shown) cholinergic receptors.

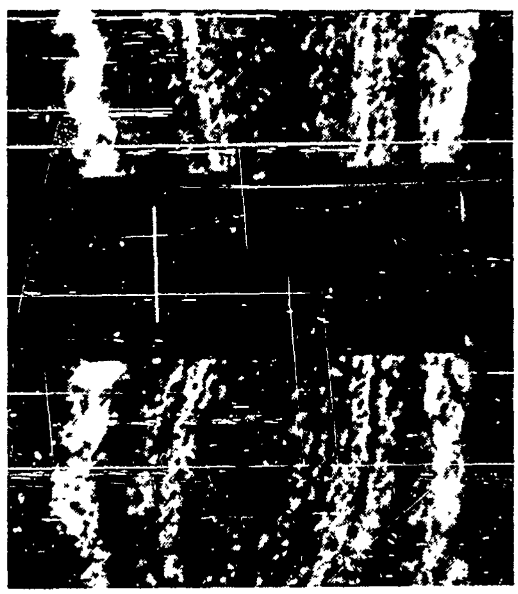
(iv.) Neurophysiological studies in our laboratory show that stimulation of NDB causes field potentials and unit activity in the appropriate bulb layers.

Thus, OB is a purely cholinoceptive target whose cholinergic innervation derives from one extrinsic source and terminates in distinct anatomical layers containing all known cholinergic markers. Manipulations of or changes in NDB can be directly correlated with changes of cholinergic markers in OB.

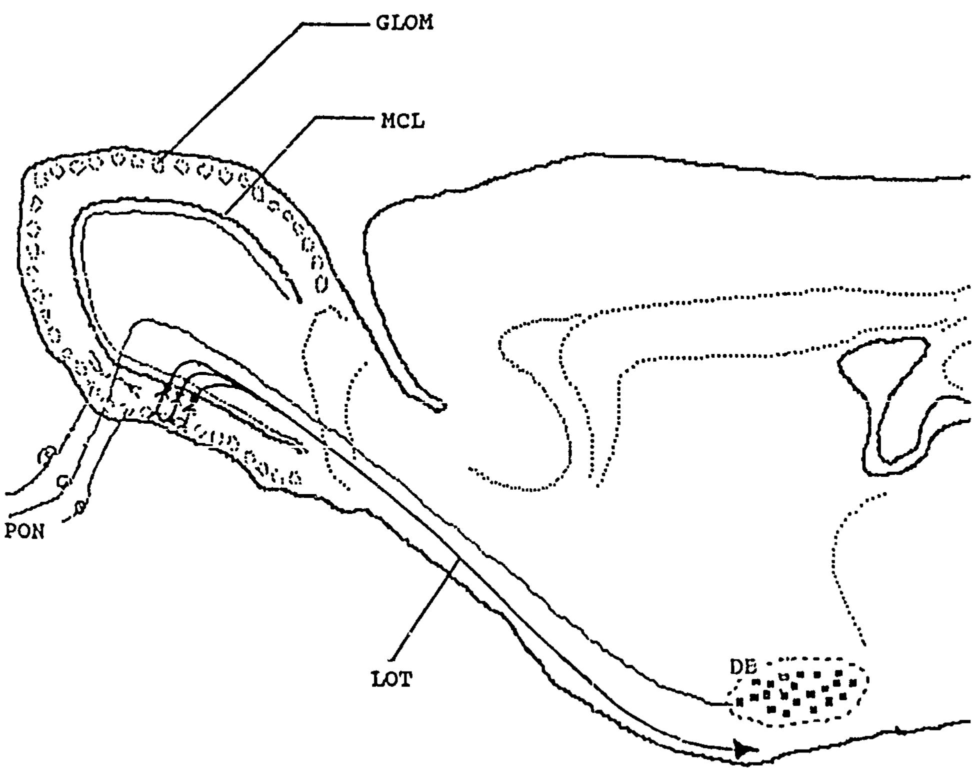


LAYERS OF THE MAIN OLFACTORY BULB:
ONL - OLFACTORY NERVE LAYER, GL - GLOMERULAR LAYER, EPL - EXTERNAL PLEXIFORM LAYER, ML - MITRAL CELL LAYER, IPL - INTERNAL PLEXIFORM LAYER, GCL - GRANULE CELL LAYER, EZ - EPENDYMAL ZONE.

NORMAL CHOLINESTERASE PATTERNS IN OLFACTORY BULB.

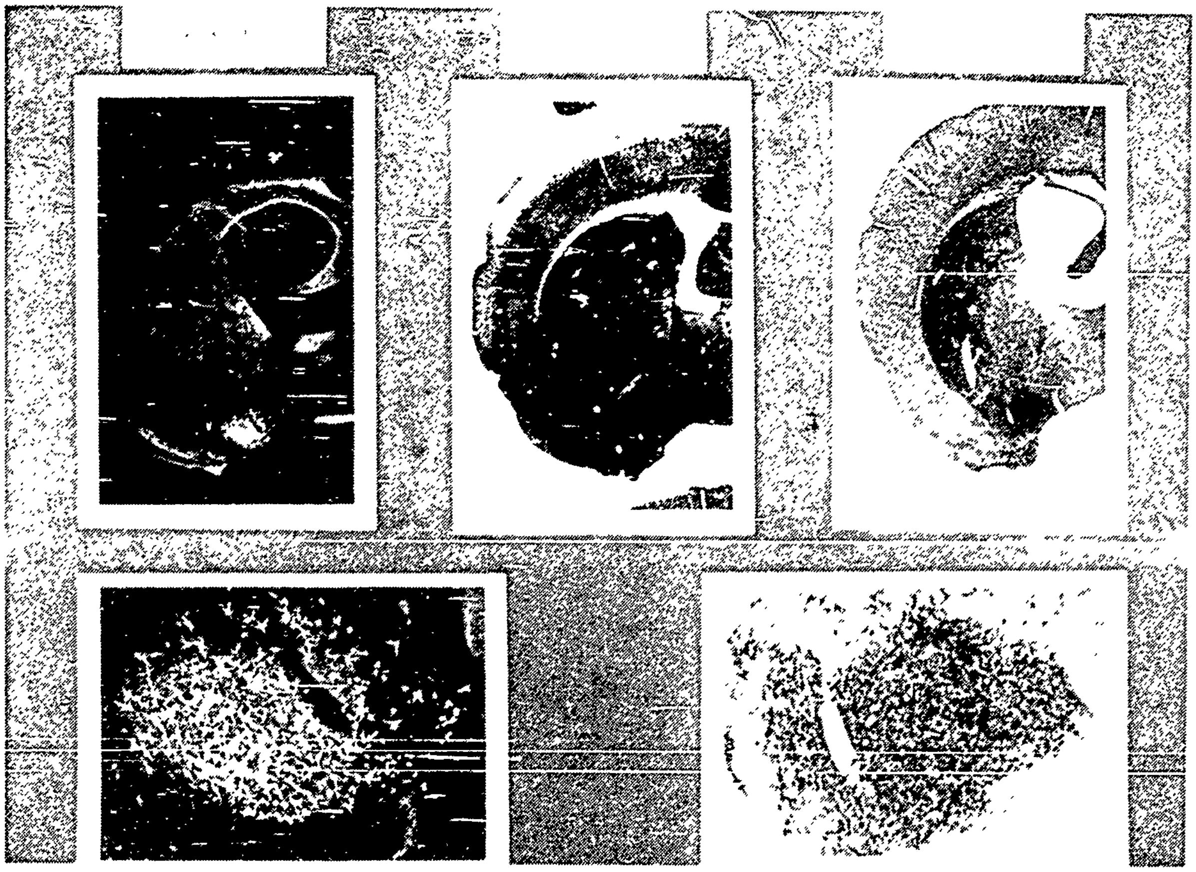


COMPUTER MEASUREMENT OF
CHOLINESTERASE DENSITY PROFILE.



TOP. IONTOPHORETIC INJECTION OF WGA-HRP AND ANTEROGRADE TRANSPORT TO THE OLFACTORY BULB.

BOTTOM. SCHEMATIC DIAGRAM OF DIAGONAL BAND -> OLFACTORY BULB PROJECTION.



A-1091

TOP ILLUSTRATION

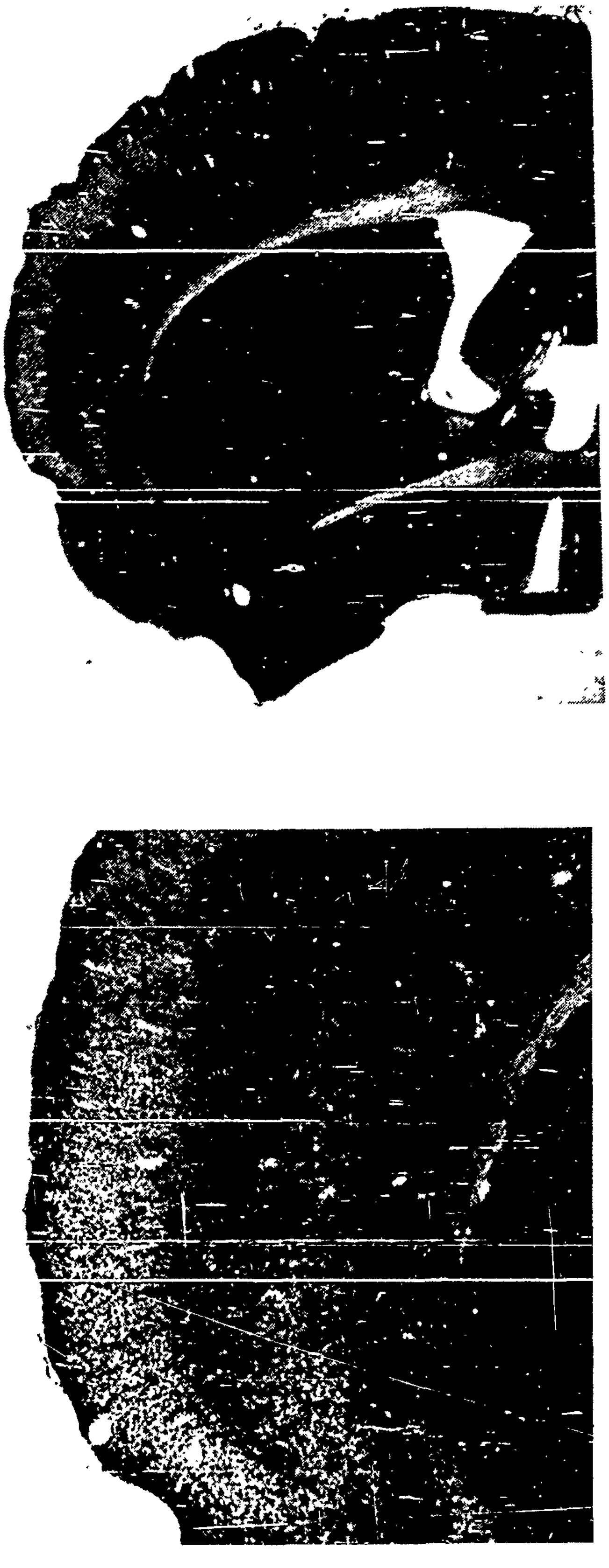
LEFT PANELS: RETROGRADE LABELING OF NDB NEURONS BY WGA-HRP INJECTION IN OB.

CENTER PANEL: ACHE STAINING AT SAME LEVEL AS THAT IN THE WGA-HRP SECTION. NOTE ACHE STAINING IS TOO DENSE TO ALLOW VISUALIZAION OF NEURONS IN NDB.

RIGHT PANELS: ACHE POSITIVE NEURONS IN NDB VISUALIZED BY PARTIAL INHIBITION OF ACHE WITH DFP (DIISOPROPYL FLUOROPHOSPHATE). NOTE CORRESPONDENCE BETWEEN RETROGRADELY LABELED NEURONS (BOTTOM LEFT) AND ACHE POSITIVE NEURONS (BOTTOM RIGHT). A SIMILAR CORRESPONDENCE IS OBTAINED WHEN CHOLINERGIC NEURONS IN NDB ARE STAINED BY MONOCLONAL ANTIBODIES TO CHOLINE ACETYLTRANSFERASE--(NOT ILLUSTRATED).

LOWER ILLUSTRATION

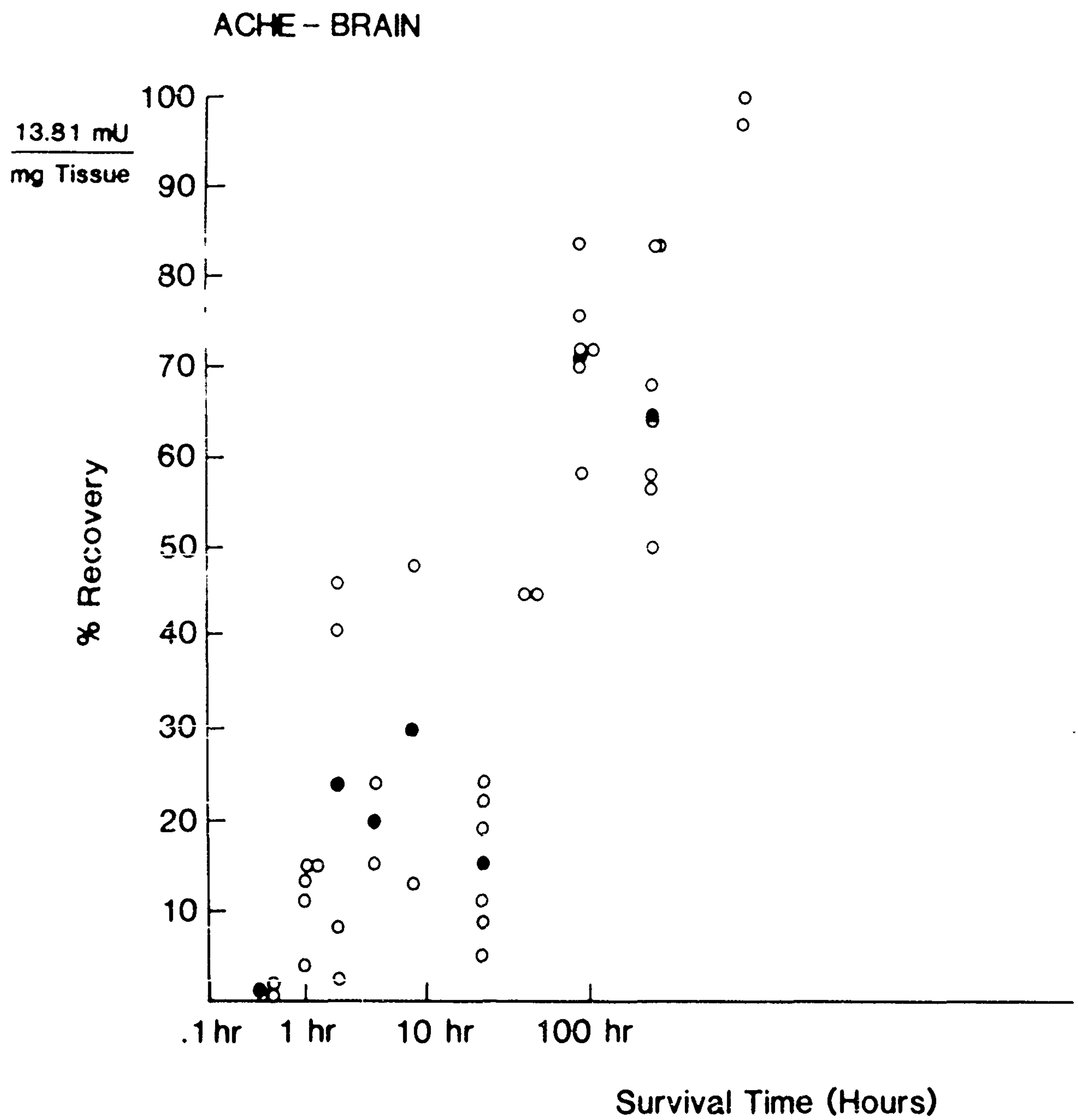
REDUCTION OF ACHE STAINING IN RIGHT BULB FOLLOWING NEUROTOXIN LESION OF IPSILATERAL DIAGONAL BAND.



NORMAL CHOLINESTERASE PATTERNS IN CEREBRAL CORTEX.
TOP: LOW MAGNIFICATION. BOTTOM: HIGHER MAGNIFICATION.

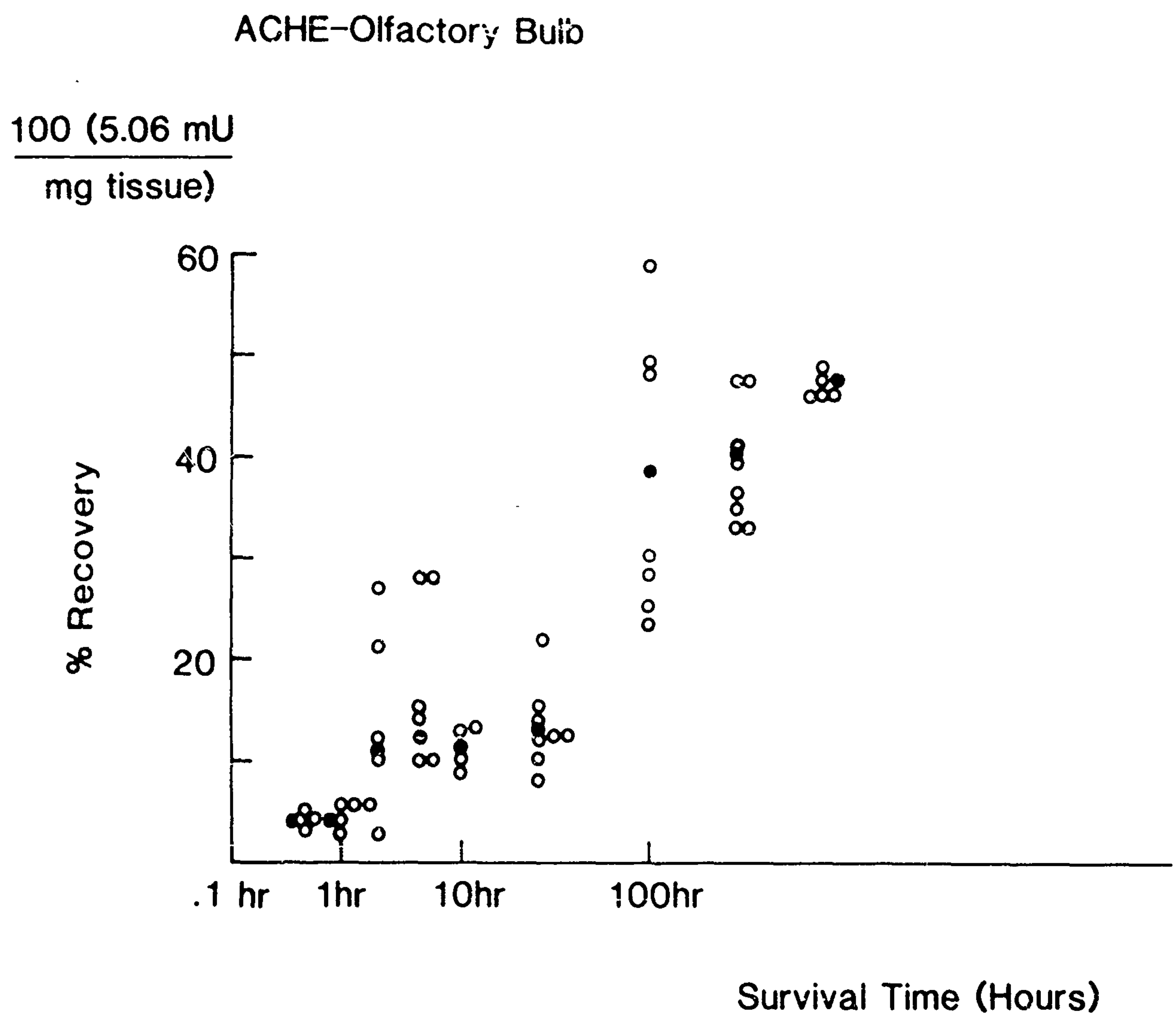
A-1093

BIOCHEMICAL OBSERVATIONS



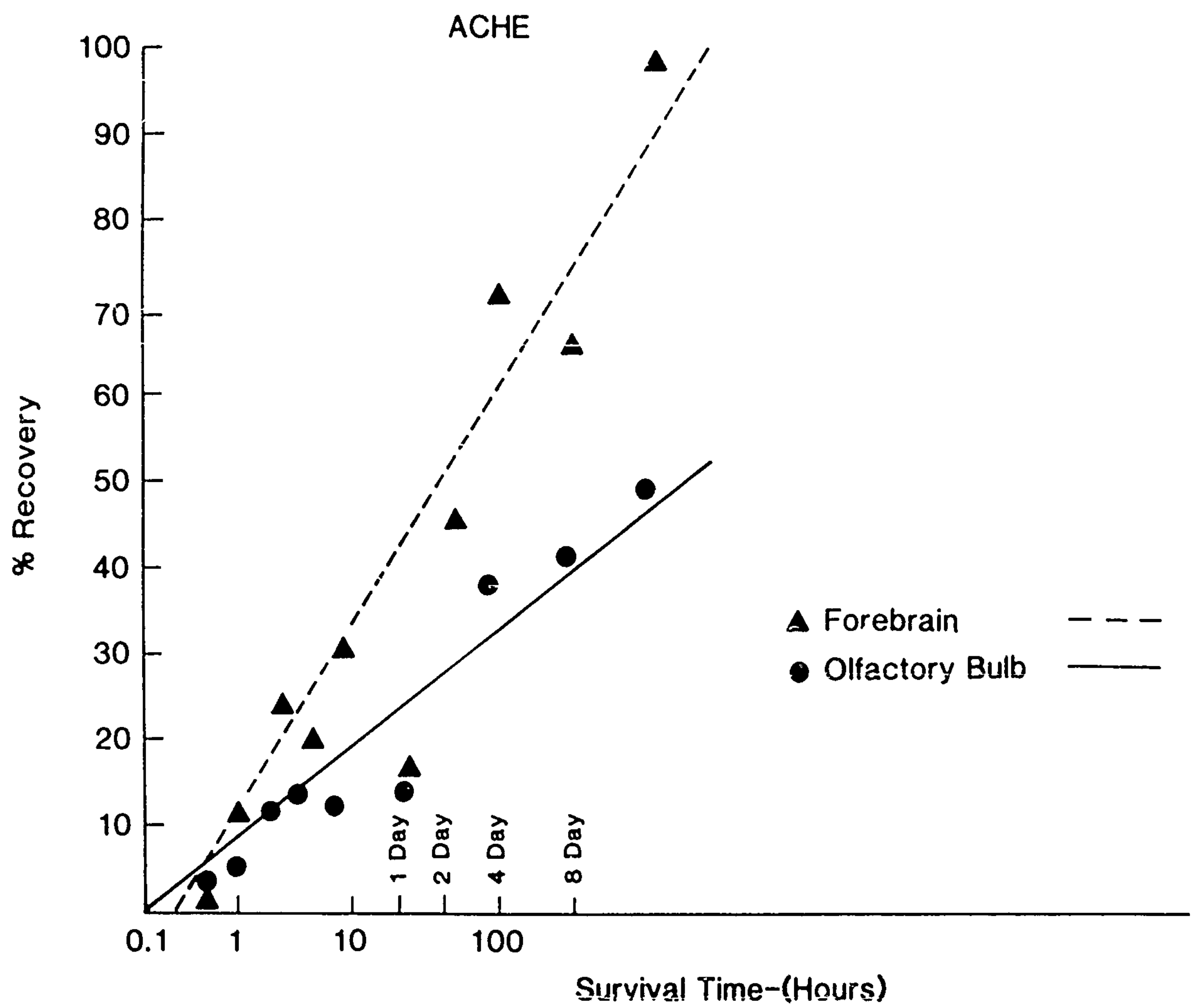
BIOCHEMICAL MEASURES OF ACHE RECOVERY IN OLFACTORY BULB FOLLOWING A SINGLE CHALLENGE OF 0.7LD50 SOMAN. SURVIVAL TIMES RANGE FROM 45 MINUTES TO 16 DAYS. OPEN CIRCLES ARE INDIVIDUAL CASES; FILLED CIRCLES ARE MEAN VALUES FOR EACH RECOVERY TIME.

A-1094



BIOCHEMICAL MEASURES OF ACHE RECOVERY IN FOREBRAIN (CORTEX & BASAL FOREBRAIN) FOLLOWING A SINGLE CHALLENGE OF 0.7LD50 SOMAN. SURVIVAL TIMES RANGE FROM 45 MINUTES TO 16 DAYS. OPEN CIRCLES ARE INDIVIDUAL CASES; FILLED CIRCLES ARE MEAN VALUES FOR EACH RECOVERY TIME.

A-1095



COMPARISON OF BIOCHEMICAL RECOVERY CURVES FOR FOREBRAIN (FILLED TRIANGLE) VERSUS OLFACTORY BULB (FILLED CIRCLE). FOREBRAIN RECOVERS ALMOST TWICE AS FAST AS OLFACTORY BULB.

FOREBRAIN SAMPLE CONTAINS BOTH CHOLINOCEPTIVE AND CHOLINERGIC NEURON CONTAINING (CAUDOPUTAMEN, BASAL FOREBRAIN) COMPARTMENTS. WE HYPOTHESIZED THAT THE CHOLINERGIC COMPARTMENT RECOVERS MOST RAPIDLY AND, BECAUSE IT CONTAINS MORE ACHE, MASKS A SLOWER RECOVERY IN THE CORTICAL CHOLINOCEPTIVE AREA. THIS HYPOTHESIS IS TESTED (RIGHT) BY MEASURING ACHE RECOVERY IN CORTEX AND BULB IN HISTOCHEMICALLY STAINED SECTIONS.

HISTOCHEMICAL OBSERVATIONS

1 day

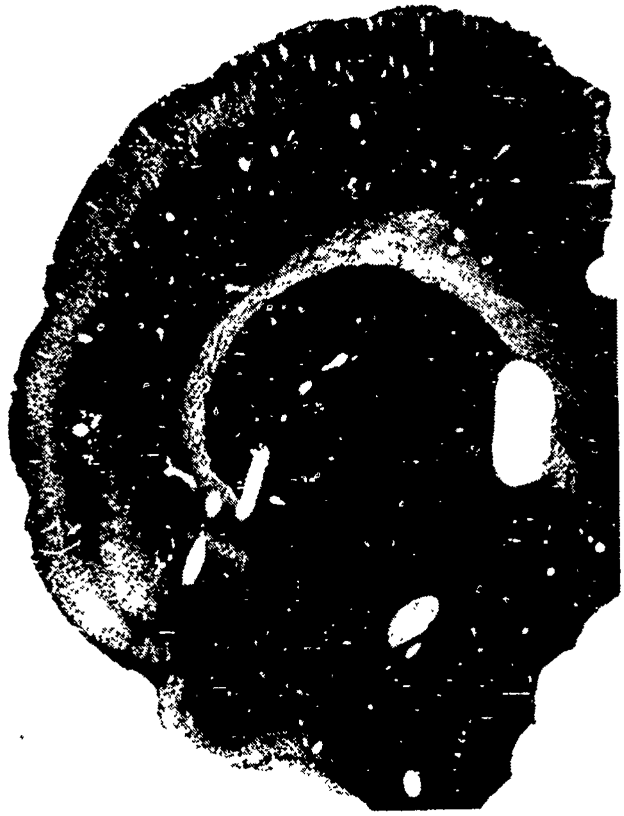
4 days

A-1007

16 days

15 days

A-1098



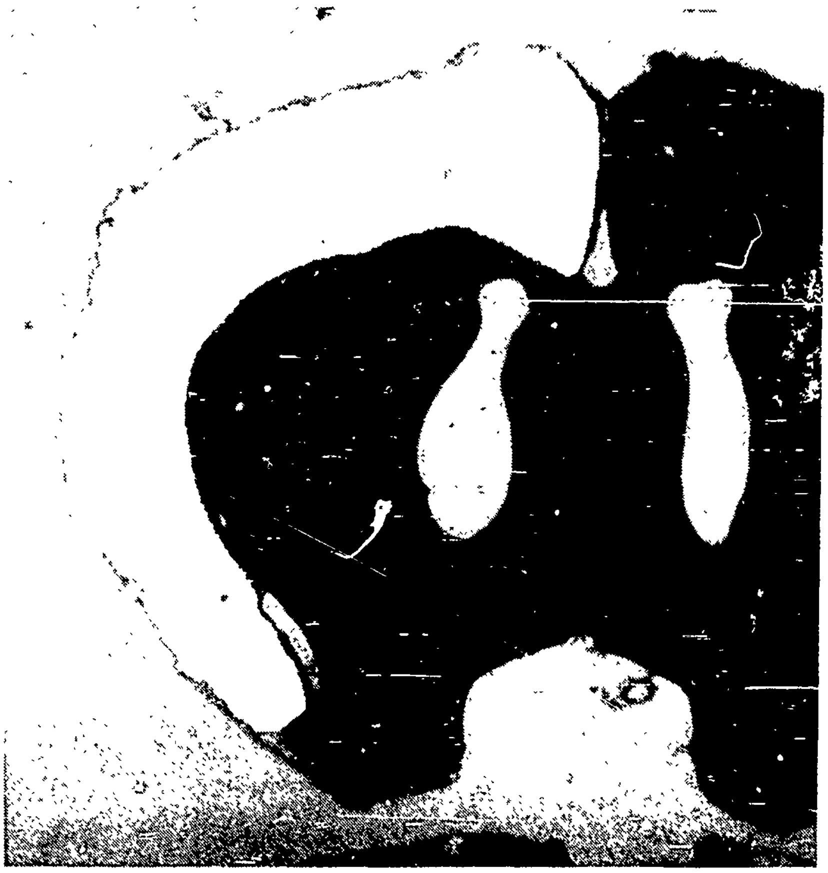
69 days

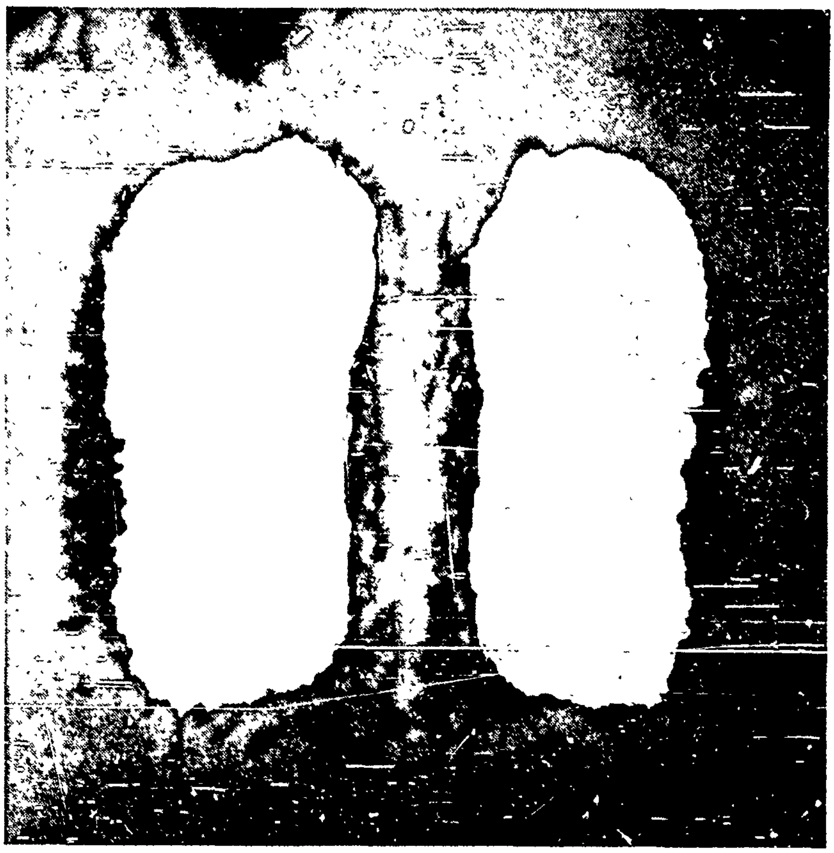
RECOVERY OF CHOLINESTERASE PATTERNS FOLLOWING SOMAN EXPOSURE

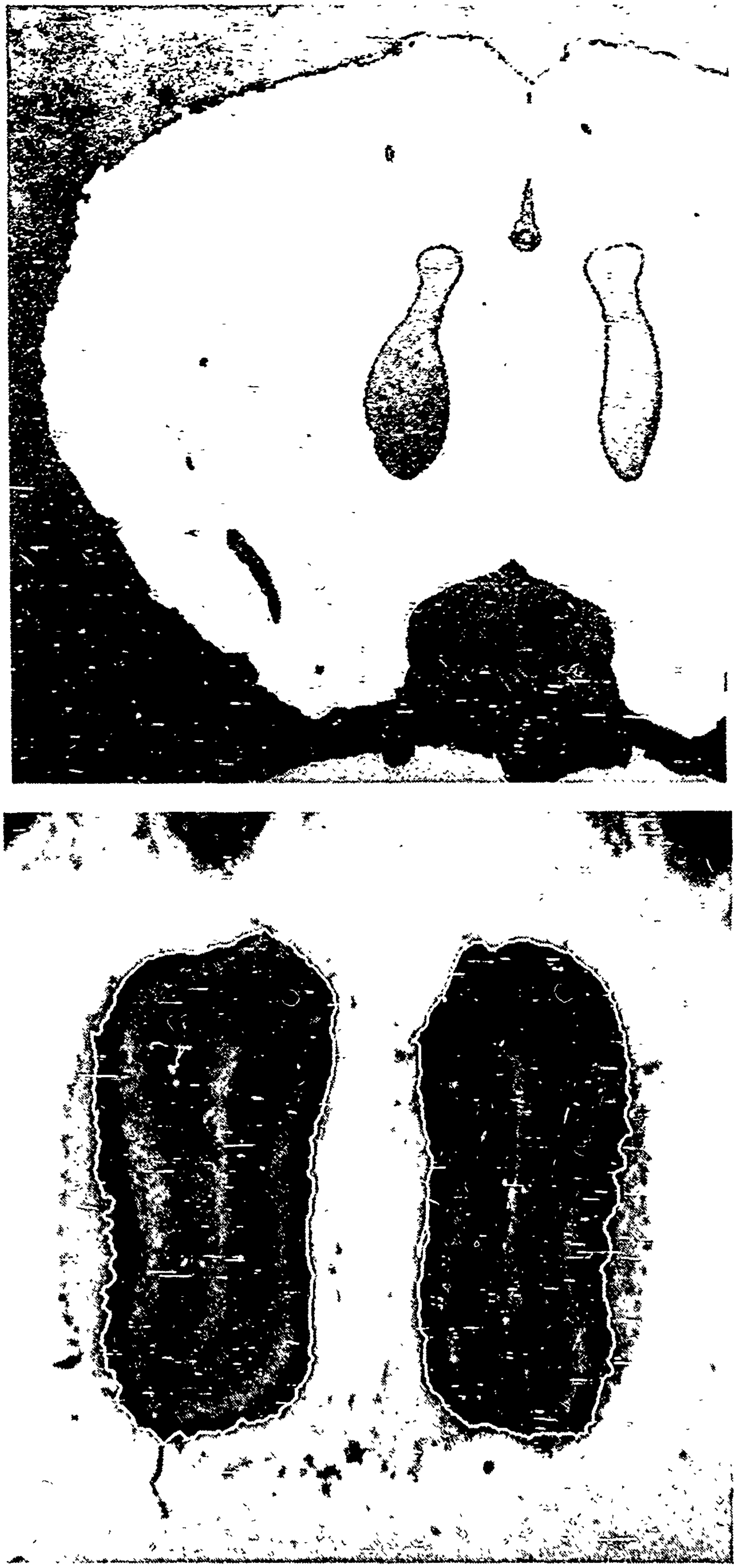
A-1099

IMAGE ANALYSIS DENSITOMETRY

A-1100







AUTOMATED ANALYSIS OF CHOLINESTERASE DENSITY. THE DIGITIZED IMAGE IS AUTOMATICALLY SEGMENTED TO DELINEATE OUTER BOUNDARIES. MANUAL EDITING IS THEN USED TO OUTLINE ANATOMICAL STRUCTURES. THE IMAGE ANALYSIS SYSTEM IS THEN ABLE TO COMPUTE THE TOTAL OPTICAL DENSITY UNDER THE SELECTED AREA OF THE SECTION.

A-1102

SUMMARY

AChE Recovery Following Single Soman Challenge

CASE	SURVIVAL (DAYS)	RECOVERY CORTEX	RECOVERY BULB
SM-073	15	40%	30%
SM-070	16	06%	10%
SM-225	20	70%	88%
SM-224	20	77%	66%
SM-139	40	61%	64%
SM-096	69	96%	74%
SM-086	87	87%	96%

Integrated optical densities and areas of experimental and control sections were measured using the automated image analysis technique illustrated at left. For each case, 7 - 10 sections were processed. Standard errors of these measurements are about 5% of the measured values.

A background level was determined using a preparation in which cholinesterase was completely abolished. AChE intensity was then calculated as (Density/Area-Background). The percent reduction reported here is calculated against a control (not Soman treated) brain processed concurrently with the experimental brain.

CONCLUSION

Soman can cause pronounced, selective, long term reductions of AChE in cortical structures. Cortical cholinergic synapses may be particularly vulnerable to Soman's actions.

REFRACTORINESS OF THE MEDULLA-PONS AND DIAPHRAGM TO ACCUMULATION OF ACETYLCHOLINE FOLLOWING AN LD_{50} OF SOMAN

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TO STUDY THE MECHANISMS OF THE ACUTE TOXICITY OF SOMAN, ACETYLCHOLINE CONCENTRATION WAS MEASURED IN EIGHT REGIONS OF THE BRAIN AND IN THE DIAPHRAGM OF THE MOUSE. SPRAGUE DAWLFY ICR MICE FROM HARLEN WERE GIVEN AN I.V. LD_{50} OF SOMAN AND SACRIFICED EITHER 1, 2, 7 OR 10 MINUTES LATER USING MAGNETIC MODE MICROWAVE IRRADIATION FOR 280 MSEC FOR THE HEAD OR 10 TO 240 MINUTES LATER USING A TIME OF 600 MSEC FOR THE DIAPHRAGM. AFTER DISSECTION OF THE TISSUE ACETYLCHOLINE WAS MEASURED BY PYROLYSIS GAS CHROMATOGRAPHY. THE ACCUMULATION RATE WAS CALCULATED BY PLOTTING TIME AGAINST THE ACETYLCHOLINE CONCENTRATION. THE ACCUMULATION RATES GIVEN IN NMOL/G/MIN WERE: HIPPOCAMPUS 3.7, CEREBRAL CORTEX 2.8, MIDBRAIN 2.7, THALAMUS 2.4, CORPUS STRIATUM 1.7, CEREBELLUM 1.6, HYPOTHALAMUS, 1.6, MEDULLA-PONS 0 AND DIAPHRAGM 0. THE MEDULLA-PONS AND DIAPHRAGM WERE THE ONLY TISSUES IN THE STUDY THAT DID NOT RESPOND TO SOMAN WITH A STRONG GENERALIZED INCREASE IN ACETYLCHOLINE CONCENTRATION. THE MEDULLA-PONS CAN INCREASE ITS ACETYLCHOLINE CONCENTRATION IN RESPIRED RATS AS SHOWN IN OUR STUDIES USING AN LD_{100} OF THE ORGANOPHOSPHOROUS CHOLINESTERASE INHIBITOR DICHLORVOS. THIS HIGHER DOSE IN RATS RESULTED IN A SYNTHESIS RATE FOR ACETYLCHOLINE IN NMOL/G/MIN LISTED IN ORDER OF DECREASING RATE: 1) STRIATUM 49.5; 2) HIPPOCAMPUS 27; 3) CEREBRAL CORTEX 18.5; 4) MEDULLA-PONS 14.8; 5) MIDBRAIN 7.7; 6) THALAMUS 7.2; 7) CEREBELLUM 3.2. THE RATE OF SYNTHESIS OF ACETYLCHOLINE IN THE MEDULLA-PONS WOULD NOT BE THE LIMITING FACTOR FOR ACCUMULATION OF ACETYLCHOLINE. THE MEDULLA-PONS DOES NOT ACCUMULATE MEASURABLE AMOUNTS OF ACETYLCHOLINE FOLLOWING INHIBITION OF ACETYLCHOLINESTERASE BY AN LD_{50} OF SOMAN. HOWEVER, GREATER DOSES OF A CHOLINESTERASE INHIBITOR WILL RESULT IN ACETYLCHOLINE ACCUMULATION. THE MEDULLA-PONS AND THE DIAPHRAGM, BOTH CRITICAL AREAS IN THE LETHAL EFFECTS OF CHOLINESTERASE INHIBITORS, ARE MORE RESISTANT TO ACETYLCHOLINE ACCUMULATION THAN OTHER BRAIN REGIONS STUDIED. ACETYLCHOLINESTERASE APPEARS TO HAVE A MORE LIMITED ROLE IN THE TERMINATION OF THE ACTION OF ACETYLCHOLINE IN THE MEDULLA-PONS THAN IN OTHER AREAS OF THE BRAIN. SINCE THE DIAPHRAGM ALSO DOES NOT READILY ACCUMULATE ACETYLCHOLINE FOLLOWING INHIBITION OF CHOLINESTERASE, DIFFUSION OF THE NEUROTRANSMITTER MAY BE AN IMPORTANT FACTOR IN TERMINATING THE LOCAL ACTION OF ACETYLCHOLINE IN BOTH THESE SITES.

INTRODUCTION

THE MAJOR POSTULATED MECHANISM OF ACTION OF SOMAN IS INHIBITION OF THE ENZYME ACETYLCHOLINESTERASE WITH THE SUBSEQUENT ACCUMULATION OF ACETYLCHOLINE. SINCE THE ACCURATE ESTIMATION OF IN VIVO LEVELS OF ACETYLCHOLINE DEPENDS UPON RAPID UNIFORM INACTIVATION OF BOTH THE SYNTHETIC AND DEGRADATION ENZYMES, THIS STUDY ON ACETYLCHOLINE CONCENTRATION WAS CARRIED OUT USING A MICROWAVE INSTRUMENT THAT WAS SPECIFICALLY DESIGNED AND BUILT TO ACCOMPLISH THIS.

METHODS

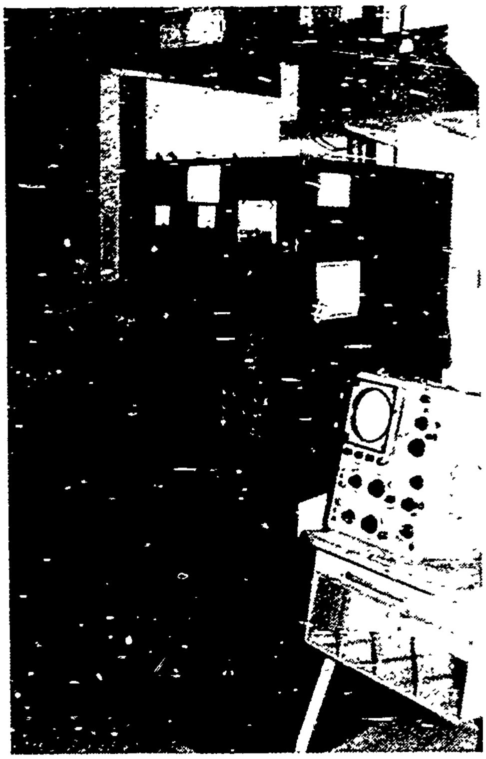
MALE MICE WERE INJECTED WITH AN LD_{50} OF SOMAN AND THEN SACRIFICED BY MICROWAVE IRRADIATION AT TIME INTERVALS OF 0 TO 240 MINUTES FOLLOWING TREATMENT. THE MOUSE WAS DISSECTED AND ACETYLCHOLINE WAS ANALYZED BY PYROLYSIS GAS CHROMATOGRAPHY.

RESULTS

FOLLOWING AN LD_{50} OF SOMAN, ACETYLCHOLINE ACCUMULATED IN THE CEREBELLUM, MIDBRAIN, THALAMUS, HYPOTHALAMUS, HIPPOCAMPUS, CORPUS STRIATUM AND CEREBRAL CORTEX. NO CHANGE IN CONCENTRATION OF ACETYLCHOLINE OCCURRED IN THE MEDULLA-PONS OR THE DIAPHRAGM.

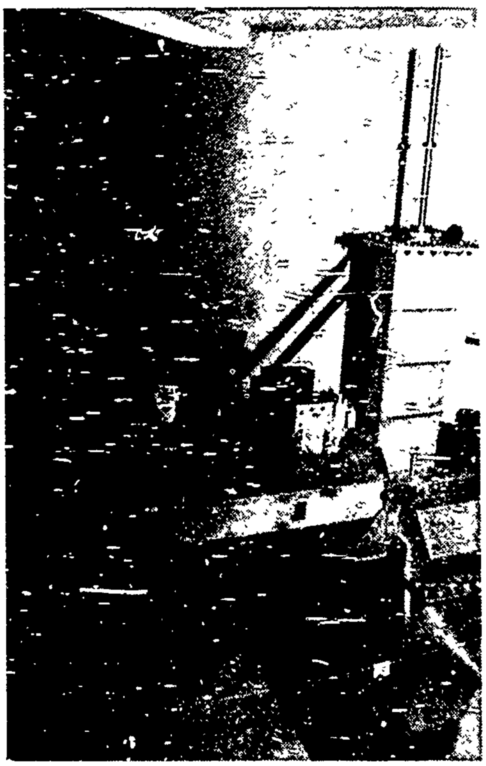
IN THE DIAPHRAGM THE ACh LEVEL REMAINED UNCHANGED AT ALL TIME INTERVALS STUDIED FOLLOWING DICA. THE LD_{50} OF SOMAN CAUSED THE 10, 120 AND 240 MINUTE ACh LEVELS TO BE LOWER THAN THE 30 AND 60 MINUTE LEVELS.

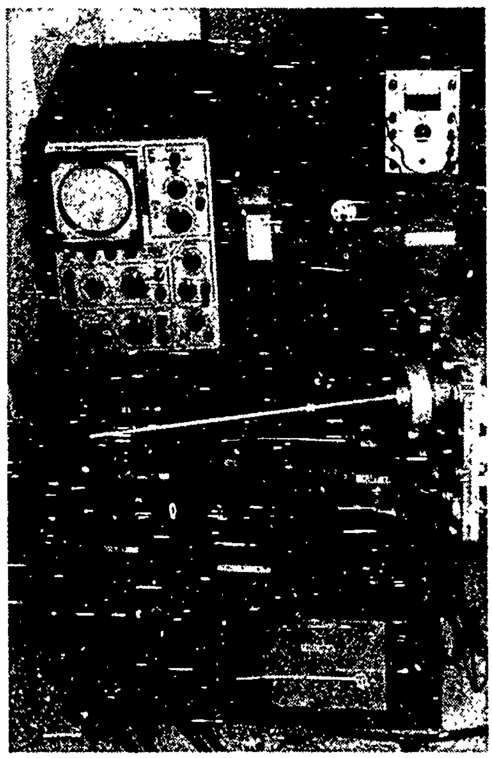
IN THE MEDULLA-PONS AN LD_{50} OF SOMAN RESULTED IN A LOWER LEVEL OF ACh AT 30 MINUTES AS COMPARED TO 10, 60 AND 120 MINUTES.



2450 MHz MICROWAVE

10 kW MICROWAVE INSTRUMENT





915 MHz MICROWAVE AND POWER SUPPLY

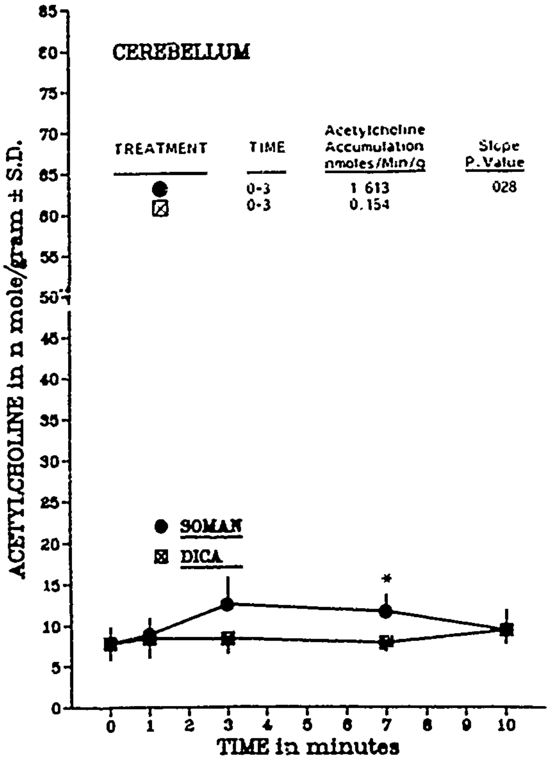


FIG. A COMPARISON OF THE TIME COURSE OF ACh CONCENTRATION FOLLOWING DICA 2.5%, 2 ml/kg I.V. AT ZERO TIME AND AND I.V. OF SOMAN LD_{50} AT ZERO TIME.
*DENOTES SIGNIFICANCE AT PVALUE OF 0 02 OR LESS.

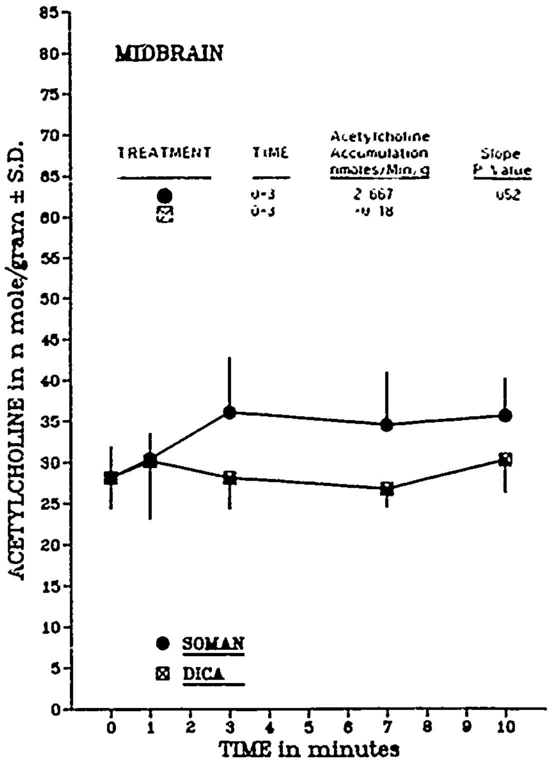


FIG. B COMPARISON OF THE TIME COURSE OF ACh CONCENTRATION FOLLOWING DICA 2.5%, 2 ml/kg I.V. AT ZERO TIME AND AN I.V. OF SOMAN LD_{50} AT ZERO TIME.
*DENOTES SIGNIFICANCE AT PVALUE OF 0.02 OR LESS.

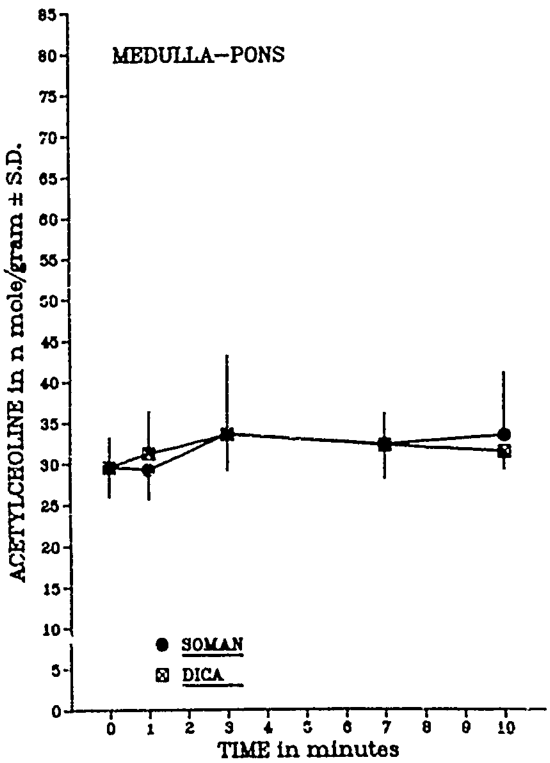


FIG. C COMPARISON OF THE TIME COURSE OF ACh CONCENTRATION FOLLOWING DICA 2.5%, 2 ml/kg I.V. AT ZERO TIME AND AND I.V. OF SOMAN LD_{50} AT ZERO TIME
*DENOTES SIGNIFICANCE AT PVALUE OF 0 02 OR LESS

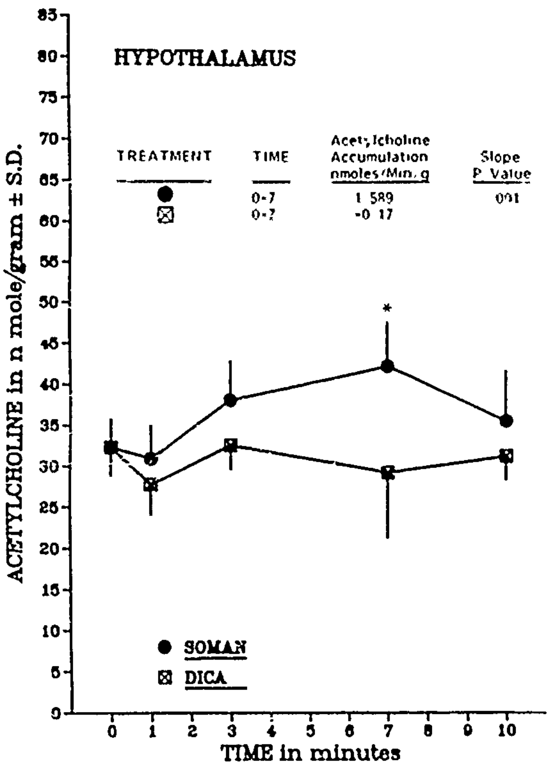


FIG D COMPARISON OF THE TIME COURSE OF ACh CONCENTRATION FOLLOWING DICA 2.5%, 2 ml/kg I.V. AT ZERO TIME AND AN I.V. OF SOMAN LD_{50} AT ZERO TIME
*DENOTES SIGNIFICANCE AT PVALUE OF 0 02 OR LESS

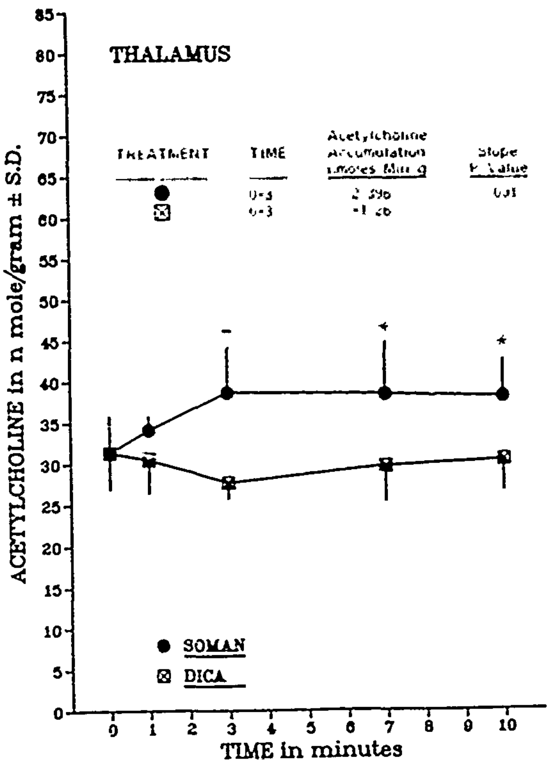


FIG E COMPARISON OF THE TIME COURSE OF ACh CONCENTRATION FOLLOWING DICA 2.5%, 2 ml/kg I.V. AT ZERO TIME AND AN I.V. OF SOMAN LD_{50} AT ZERO TIME
*DENOTES SIGNIFICANCE AT PVALUE OF 0.02 OR LESS

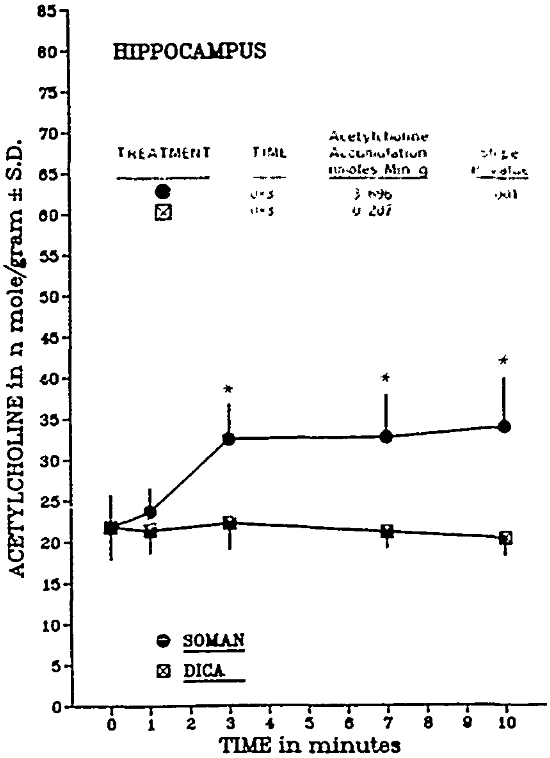


FIG F COMPARISON OF THE TIME COURSE OF ACh CONCENTRATION FOLLOWING DICA 2.5%, 2 ml/kg I.V. AT ZERO TIME AND AN I.V. OF SOMAN LD_{50} AT ZERO TIME.
*DENOTES SIGNIFICANCE AT PVALUE OF 0.02 OR LESS.

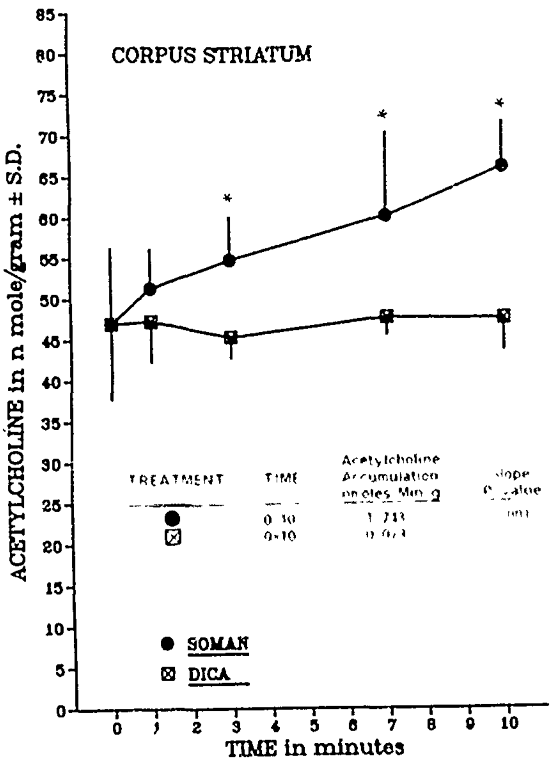


FIG G COMPARISON OF THE TIME COURSE OF ACh CONCENTRATION FOLLOWING DICA 2.5%, 2 ml/kg I.V. AT ZERO TIME AND AN I.V. OF SOMAN LD_{50} AT ZERO TIME
*DENOTES SIGNIFICANCE AT PVALUE OF 0.02 OR LESS

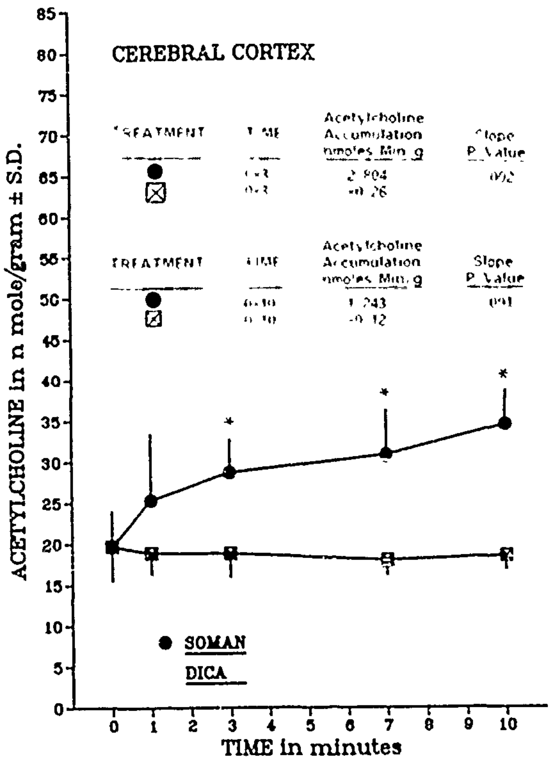


FIG H COMPARISON OF THE TIME COURSE OF ACh CONCENTRATION FOLLOWING DICA 2.5%, 2 ml/kg I.V. AT ZERO TIME AND AN I.V. OF SOMAN LD_{50} AT ZERO TIME
*DENOTES SIGNIFICANCE AT PVALUE OF 0.02 OR LESS

TABLE I

ACETYLCHOLINE CONCENTRATION nmoles/DIAPHRAGM ± SD AND RANGE ( )

IN THE MOUSE FOLLOWING SOMAN AND PROTECTIVE COMPOUNDS

TREATMENT	Time in Minutes 10	30	60	120	240
CONTROL	0.12 ± 0.02 (0.05)	0.14 ± 0.01 (0.02)	0.12 ± 0.03 (0.07)	0.10 ± 0.03 (0.05)	0.12 ± 0.05 (0.12)
DICA	0.13 ± 0.04 (0.10)	0.13 ± 0.00 (0.01)	0.11 ± 0.02 (0.05)	0.12 ± 0.02 (0.06)	0.12 ± 0.02 (C.05)
SOMAN	0.08 ± 0.02 (0.06)	9.014± 0.01 (0.02)	0.15 ± 0.03 (0.06)	0.08 ± 0.02 (0.04)	0.11 ± 0.03 (0.06)

N = 5 mice Enzyme inactivation by microwave heating adjusted between 650-800 milliseconds.

TABLE II

ACETYLCHOLINE CONCENTRATION IN nmoles/g ± SD AND RANGE ( ) IN THE MEDULLA-PONS

OF THE MOUSE FOLLOWING SOMAN AND PROTECTIVE COMPOUNDS

TREATMENT	Time in Minutes 10	30	60	120	240
CONTROL	31.9 ± 2.3 (6.7)	32.2 ± 1.7 (5.1)	32.1 ± 3.6 (9)	30.2 ± 3.3 (9.7)	30.6 • 2.6 (7.1)
DICA	29.0 ± 2.3 (7.4)	31.3 ± 3.7 (9.4)	31.3 ± 3.9 (11)	33.2 ± 3.2 (8.8)	31.6 ± 9.4 (13.2)
SOMAN	33.3 ± 3.18 (11 1)	28.0 ± 2.8 (6.8)	34.0 ± 3.1 (8.7)	34.9 ± 2.1 (6.5)	32.3 ± 3.3 (7.9)

N = 5 mice Enzyme Inactivation by microwave 330 milliseconds.

ACETYLCHOLINE ACCUMULATION

IN THE BRAIN OF A VENTILATED RAT FOLLOWING

DICHLORVOS i.v. (LD_{100})*

	A_0 Concentration of ACh(nM/g)	τ	τ = O Synthesis rate (nM/g/min)	Turnover Time (min)
CEREBRAL CORTEX	19 ± 1 (7)	2	18.5	1.0
HIPPOCAMPUS	26 ± 3 (5)	1.5	27	1.0
STRIATUM	66 ± 9 (6)	2	49.5	1.3
CEREBELLUM	6 ± 0.6 (6)	2.5	3.2	1.9
MEDULLA-PONS	31 ± 3 (6)	2.5	14.8	2.1
MIDBRAIN	34 ± 2 (6)	6	7.7	4.4
THALAMUS	40 ± 2 (7)	7	7.2	5.6

NUMBER OF RATS GIVEN IN BRACKETS $k = 1/\tau$.

*Stavinoha, W.B., Modak, A.T. and Weintraub, S.T.: Rate of accumulation of acetylcholine in discrete regions of the rat brain after dichlorvos treatment. Journal of Neurochemistry, 23. 1375-1378, 1976.

DISCUSSION AND SUMMARY

IN THE RAT, AN LD_{100} OF DICHLORVOS, A CHOLINESTERASE INHIBITOR, WILL CAUSE AN ACCUMULATION OF ACETYLCHOLINE IN ALL THE BRAIN AREAS STUDIED. HOWEVER, THE RESPONSE TO INHIBITION OF ChE BY AN LD_{50} OF SOMAN, WITH THE SUBSEQUENT ACCUMULATION OF ACh FOLLOWED BY IMPAIRMENT OR ABOLITION OF CHOLINERGIC TRANSMISSION, IS MODERATED IN THE TWO COMPONENTS OF THE RESPIRATORY CHAIN STUDIED, THE DIAPHRAGM AND THE MEDULLA-PONS. AT THESE TWO SITES AN LD_{50} OF SOMAN DOES NOT CAUSE ACCUMULATION OF ACh, AND CAN CAUSE A DECREASE IN ACh IN THE DIAPHRAGM. THIS SUGGESTS THE POSSIBILITY THAT, IN THESE AREAS, HYDROLYSIS OF ACh BY THE ENZYME ChE IS NOT THE MAJOR MECHANISM FOR TERMINATING THE NEUROTRANSMITTER ACTION OF ACh. THE ACTION OF ACh MAY BE TERMINATED LOCALLY BY DIFFUSION OR TRANSPORT WITH THE ITINERANT ACh BEING DESTROYED BY ChE AT A DISTANT AREA. THIS WOULD GIVE CREDANCE TO THE HYPOTHESIS THAT PERIPHERALLY ACTING AGENTS CAN PROTECT AGAINST CENTRAL TOXICITY BY PROTECTING PERIPHERAL ChE FROM INHIBITION. THIS PRESERVED ENZYME IN THE BLOOD WOULD BE AVAILABLE TO DESTROY ACh WHICH HAS DIFFUSED FROM ITS SITE OF RELEASE. THE CRITICAL SITES FOR RESPIRATION SUCH AS DIAPHRAGM AND MEDULLA-PONS WOULD BE ABLE TO MAINTAIN BY CONTINUED DIFFUSION OR TRANSPORT DOWN A GRADIENT AN ACh CONCENTRATION COMPATIBLE WITH IMPULSE TRANSMISSION. IF ChE IN ALL AREAS IS INHIBITED THEN THE GENERALIZED ACCUMULATION OF ACh COULD PRECLUDE DIFFUSION OR TRANSPORT AS AN EFFECTIVE MECHANISM OF TERMINATING NEUROTRANSMITTER ACTION AND ALLOW EXCESSIVE ACCUMULATION OF ACh AT THE TRANSMISSION SITE AND RESULT IN DEPRESSION OF RESPIRATION.

EFFECTS OF SOMAN UPON PRIMATE DISCRIMINATION BEHAVIOR

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Southwest Foundation for Biomedical Research, San Antonio, TX 78284

A B S T R A C T

Juvenile male baboons (Papio cynocephalus) were trained on a match-to-sample discrimination task and the effects of sublethal doses of soman (O-1,2,2-trimethylpropylmethylphosphonofluoridate) upon daily performance of the task observed; depression and recovery of blood AChE activities were monitored. Soman was administered acutely to 6 baboons in doses ranging from 1 to 5 μg/kg (i. m.) in mixed random order; blood AChE levels returned to at least 80% of control between exposures. Acute exposure to soman produced behavioral effects primarily in the dose range of 4-5 μg/kg. Increases in mean session response times and errors, and decreases in percent trials worked were observed; trials to which animals responded were performed as accurately as normally, but no responses at all were made to clusters of trials (attentional deficits). The highest dose employed (5 μg/kg) produced an immediate seizure in one animal and stomach cramps 2-3 hrs post-soman in another animal; since this time, these 2 animals have experienced generalized seizures intermittently in the absence of additional soman. The occurrence of seizures was associated with pronounced increases in the occurrence of attentional deficits to the demands of the discrimination task. The remaining 4 baboons displayed no overt neurological symptoms at any dose, but did display behavioral effects, including recurring attentional deficits.

Upon completion of the acute dosing protocol, animals were allowed 4 months rest before initiation of subchronic soman exposure consisting of 5 weekly injections of soman at 3, 1, 1, 1, and 1 μg/kg, respectively. The subchronic soman exposure protocol produced a pattern of behavioral effects similar to that observed from the acute exposures.

From the results of both experiments a profile of primate neurobehavioral responses to soman is described. This profile encompasses immediate, persistent, and delayed effects. Consistent features of the profile include immediate dose-related effects of: increases in mean session response time (due to attentional deficits); increases in errors in performance; decreases in the incidence of extra inconsequential responses; and seizures (at the 5 μg/kg dose level). Attentional deficits and generalized seizures are both persistent and immediate effects. An additional delayed effect is a sudden, dramatic increase in the incidence of extra inconsequential responses which occurs weeks after termination of soman exposure and may persist for a number of weeks. Results of these studies indicate that sublethal soman exposure may produce CNS lesions which are not seriously incapacitating at the time of exposure, but which may significantly impair discrimination task performance and possibly become life-threatening at some later time.

This work supported in part by the US Army Medical Research and Development Command under Contract DAMD-17-82-C-2161.

I N T R O D U C T I O N

From the types of brain damage produced by soman (GD), it has been suggested that humans surviving soman exposure might have impairments of skilled movements, memory, cognition, autonomic regulation and psychiatric disorders (1). This study undertook to explore the effects of sublethal soman exposure upon ability of primates (Baboons) to perform a complex task which requires integration of CNS functions and measures cognitive ability, alertness, memory and focussing of attention.

M E T H O D S

Protocol 1

Six male juvenile baboons (*Papio cynocephalus*) 2-3 years of age, were trained to 90-100% accuracy of performance baseline before exposure to soman. The animals were then administered soman at 1-5 micrograms/kg in a mixed random order; blood AChE inhibition was monitored and another dose of soman was not administered until blood AChE levels returned to at least 80% of the pre-soman level (3-7 weeks generally). After completion of these exposures, animals were maintained on daily task performance without treatment for 4 months before initiation of Protocol 2. (For further details see Reference 2).

Protocol 2

After 4 months recovery, the same 6 baboons were exposed to soman in a subchronic fashion. After baselines were re-established, all animals received an initial dose of 3 μg/kg, followed at weekly intervals by doses of 1 μg/kg for 4 weeks. After termination of exposure, behavioral recovery was monitored for 6 weeks. (For further details see Reference 3).

R E S U L T S

Protocol 1. Acute Soman Exposure.

Objective:

To determine behaviorally-effective, sublethal dose range of i. m. soman for the juvenile Baboon.

1-a. Acute Soman Dose-effects upon Discrimination Behavior and Blood AChE (Figure 1).

Blood AChE Activity. Whole blood AChE activity was inhibited linearly, with 5 μg/kg soman producing approximately 70% inhibition (Figure 1).

Match-to-Sample Discrimination Behavior. Acute soman at 1-3 μg/kg had no effect upon baboon behavioral performance although blood AChE activity was inhibited up to 50%. Acute soman at 4 and 5 μg/kg (AChE inhibition 50-70%) produced effects upon discrimination behavior including:

- Decrease in percent trials attempted (Figure 1);
- Increase in mean session response time (Figure 1);
- Decline in number of extra inconsequential responses (Figure 1);
- Delayed increase in number of extra responses during recovery weeks (data not shown).

1-b. Persistent Attentional Deficits to Task after Acute Soman (Figure 2).

Soman at 4-5 μg/kg produced significant, persistent changes in a baboon's pattern of responding to the repetitive demands of the Discrimination Task. Normally, all stimuli presented during the 2-hr behavioral session are responded to correctly within approximately 2 sec; however, after soman, some trials are still responded to normally, but clusters of trials are ignored - (i.e., no responses are made to either the side stimuli within 20 sec or to center stimuli within 30 sec). These ignored trials represent deficits in attention to the task.

After administration of 4 - 5 μg/kg acute soman, baboons exhibited attentional deficits for 1-2 days, then appeared to recover (Figure 2). However, after this level of exposure, similar attentional deficits occurred on a random, intermittent basis in the absence of additional soman (for example, Figure 5).

Protocol 2. Subchronic Soman Exposure.

Objective:

To evaluate possible cumulative effects of subchronic exposure to soman at doses below the acute behaviorally-effective threshold dose.

2-a. Subchronic Soman Inhibition of Blood AChE Activities (Figure 3).

The subchronic soman protocol produced and maintained some degree of inhibition of both plasma and RBC AChE throughout the 5 weeks of exposure. After termination of exposures, activities of both fractions returned to control range within 3-4 weeks (Figure 3).

2-b. Effects of Subchronic Soman Upon Discrimination Task (Figure 4).

The baboons in this study exhibited a significant degree of behavioral tolerance to the subchronic soman protocol employed. Even though blood AChE was inhibited throughout (Figure 3), only the initial 3 μg/kg dose of soman affected behavior.

- The percentage of trials attempted decreased after the initial 3 μg/kg dose, but was not affected by subsequent 1 μg/kg dose, (Figure 4).
- Mean session response time increased in response to the initial 3 μg/kg dose; however, subsequent 1 μg/kg doses produced only increased variability (Figure 4).
- Accuracy of performance was not affected by subchronic soman exposure; all animals maintained their pre-soman 90-100% level of correct responding throughout the subchronic exposure (Figure 4).

2-c. Relationship of Attentional Deficits to Seizures (Figure 5).

For 2 baboons which have experienced intermittent generalized seizures since receiving 5 μg/kg soman, the onset of attentional deficits to the behavioral task is associated with the occurrence of seizures (Figure 5). Of the remaining 3 baboons, 2 exhibit attentional deficits intermittently, but have not been observed to experience generalized seizures, suggesting that the attentional deficits might reflect focal brain seizure activity. One baboon suddenly began exhibiting attention deficits and after 3 days of this pattern died overnight. One animal has not exhibited attentional deficits (or experienced seizures) to date.

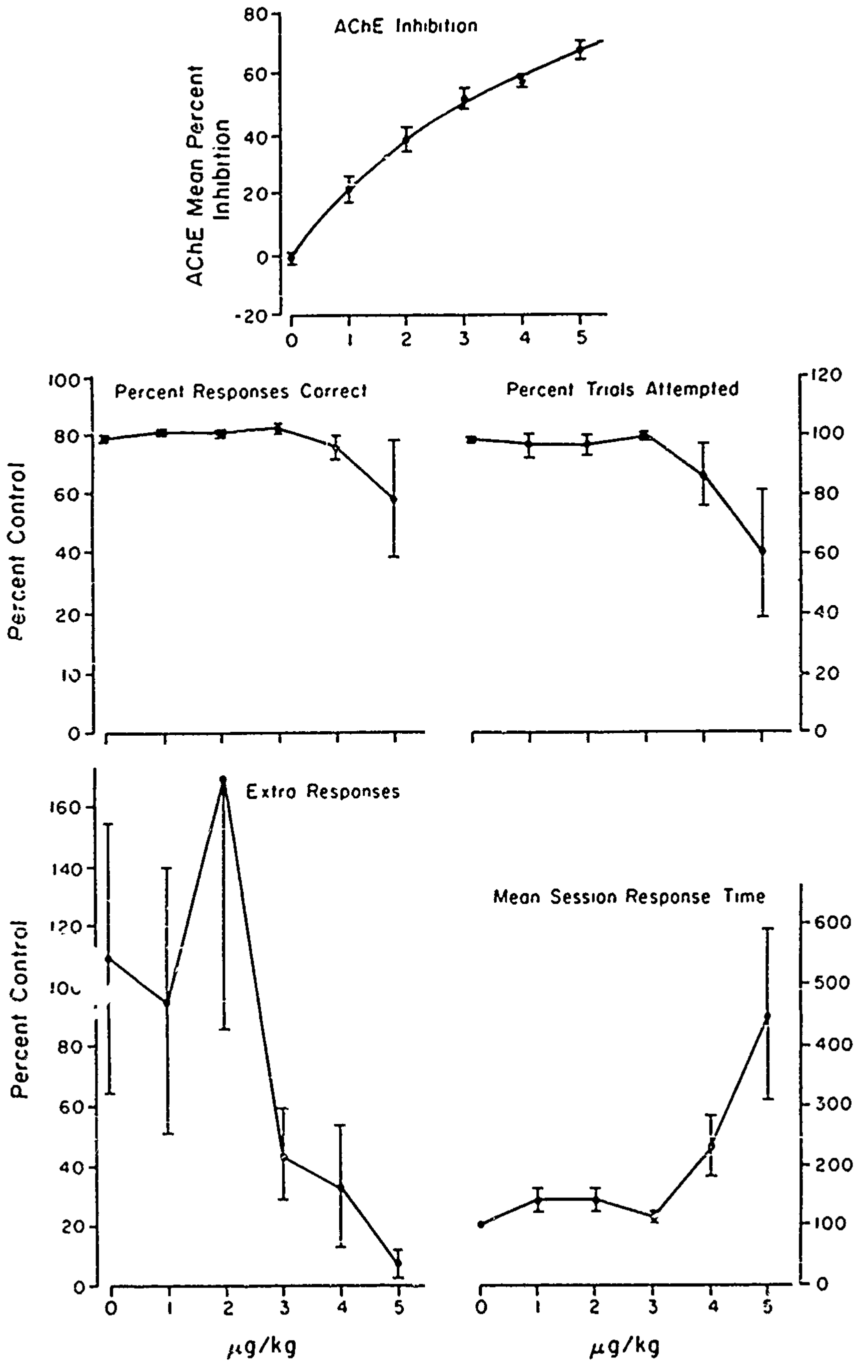

Figure 1. Dose-effects of acute soman upon whole blood AChE activity and match-to-sample discrimination task performance immediately after soman. Group means and S. E. M. for 6 baboons are plotted as a function of the dose of soman in μg/kg.

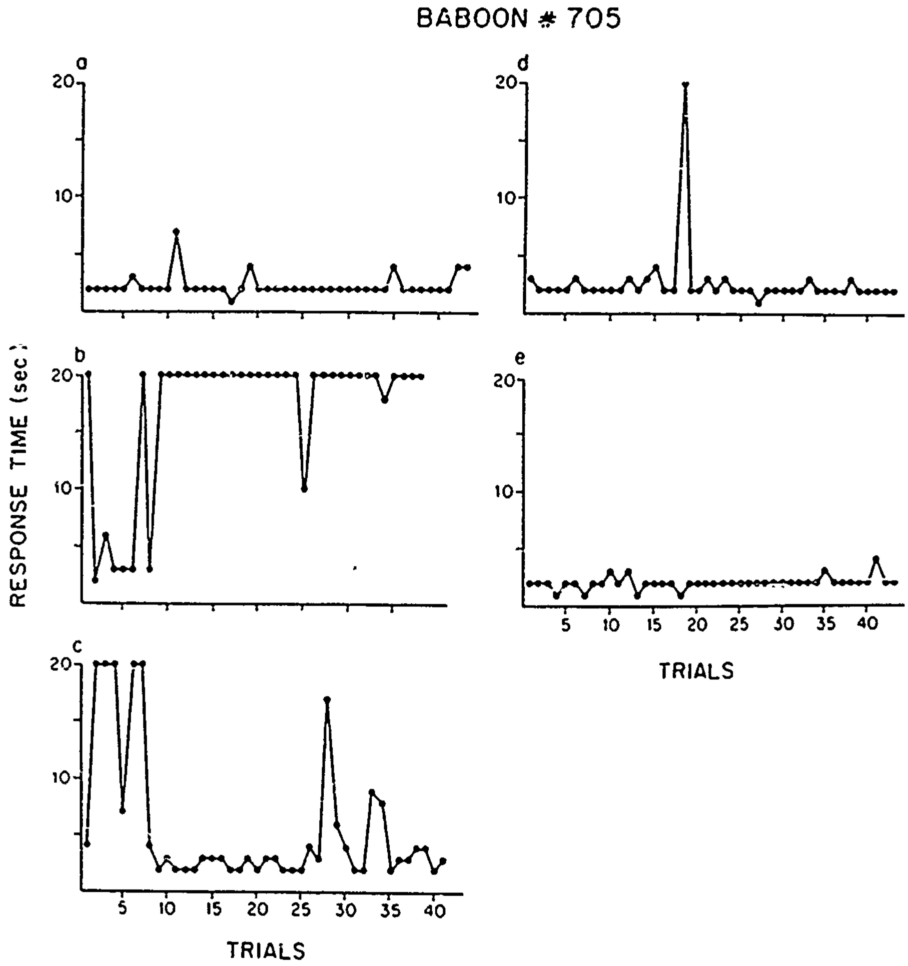

Figure 2. Effects of 5 μg/kg soman upon trial-by-trial response times of Baboon 705 performing the match-to-sample discrimination task. (a) Day before soman treatment; (b) Session initiated within 20 min after soman injection (whole blood AChE inhibited 63%; no overt neurological symptoms); (c) 24 hrs after soman; (d) 48 hrs after soman; (e) 120 hrs after soman.

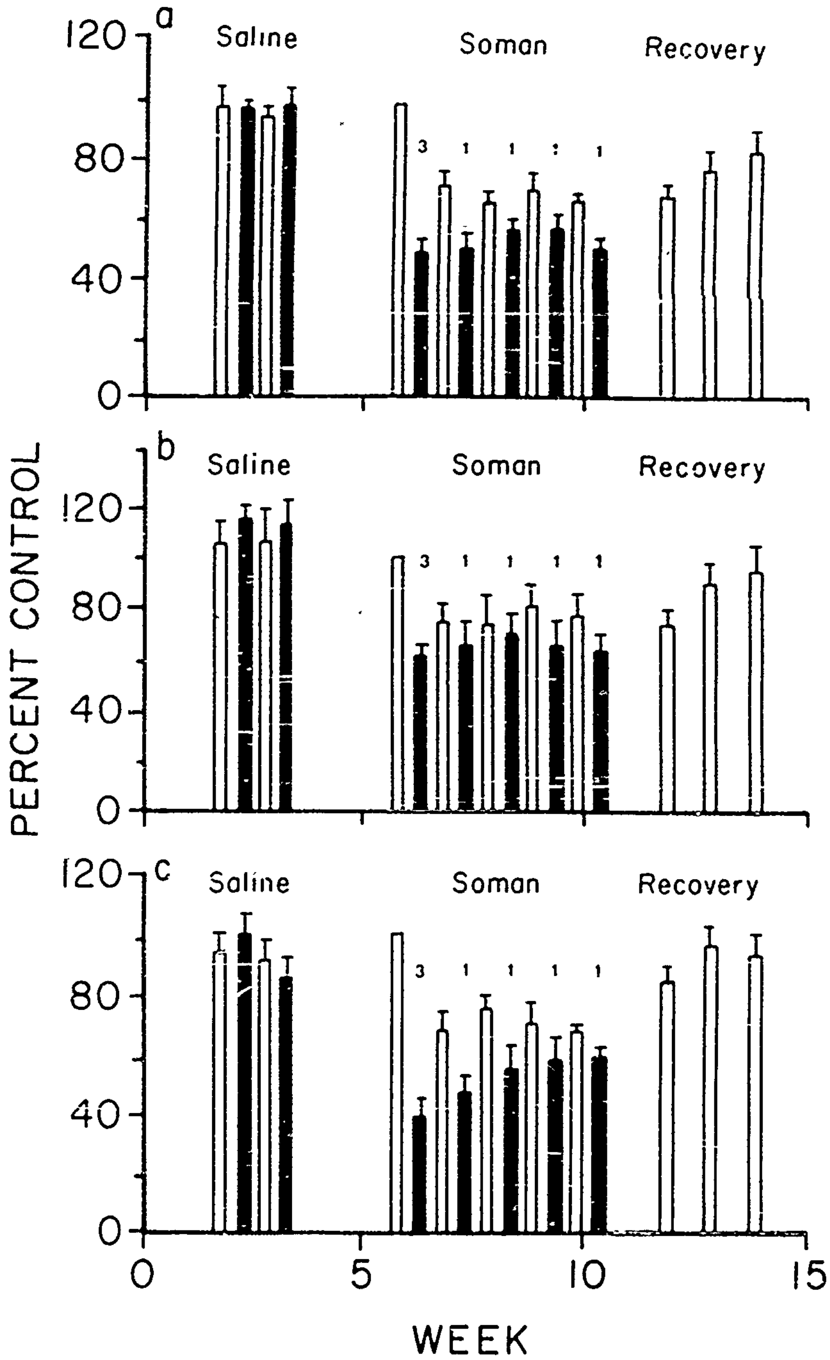

Figure 3. Effects of subchronic soman exposure protocol upon baboon blood AChE activities. Blood samples were obtained immediately before the soman or saline injections preceding behavioral sessions, and within 20 min after completion of the 2-hr sessions. During recovery periods, blood was sampled after completion of the behavioral sessions at weekly intervals. Values shown are group means and S. E. M. for (a) whole blood, (b) erythrocytes, and (c) plasma, all expressed as percent control. Control (100%) level taken as value immediately preceding the intial 3 g/kg dose of of soman for each individual animal.

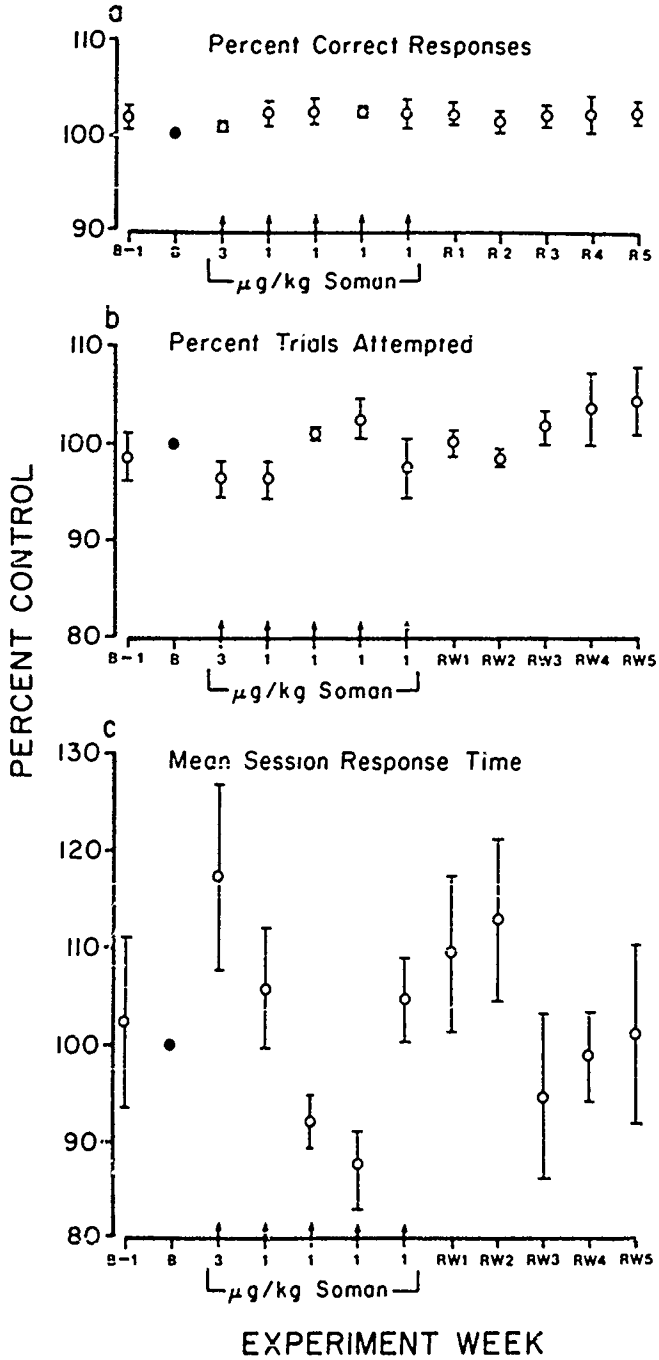

Figure 4. Effects of subchronic soman exposure protocol upon match-to-sample discrimination task parameters. The mean of the 5 daily sessions during the week for each animal was compared to that animal's mean for the baseline week and expressed as percent control; these values were then averaged across subjects and the mean and S. E. M. values plotted as a function of experimental week. Baseline week (100% Control) was the week immediately preceding the initial subchronic (3 μg/kg) dose of soman. Statistical significance was examined by means of a Repeated Measures ANOVA comparing Experimental Week with Baseline Week. (a) Percent correct responses ($F=1.042$; $P=.428$); (b) Percentage of trials worked ($F=1.234$; $P=.293$); (c) Mean session response time ($F=1.584$; $P=.137$);

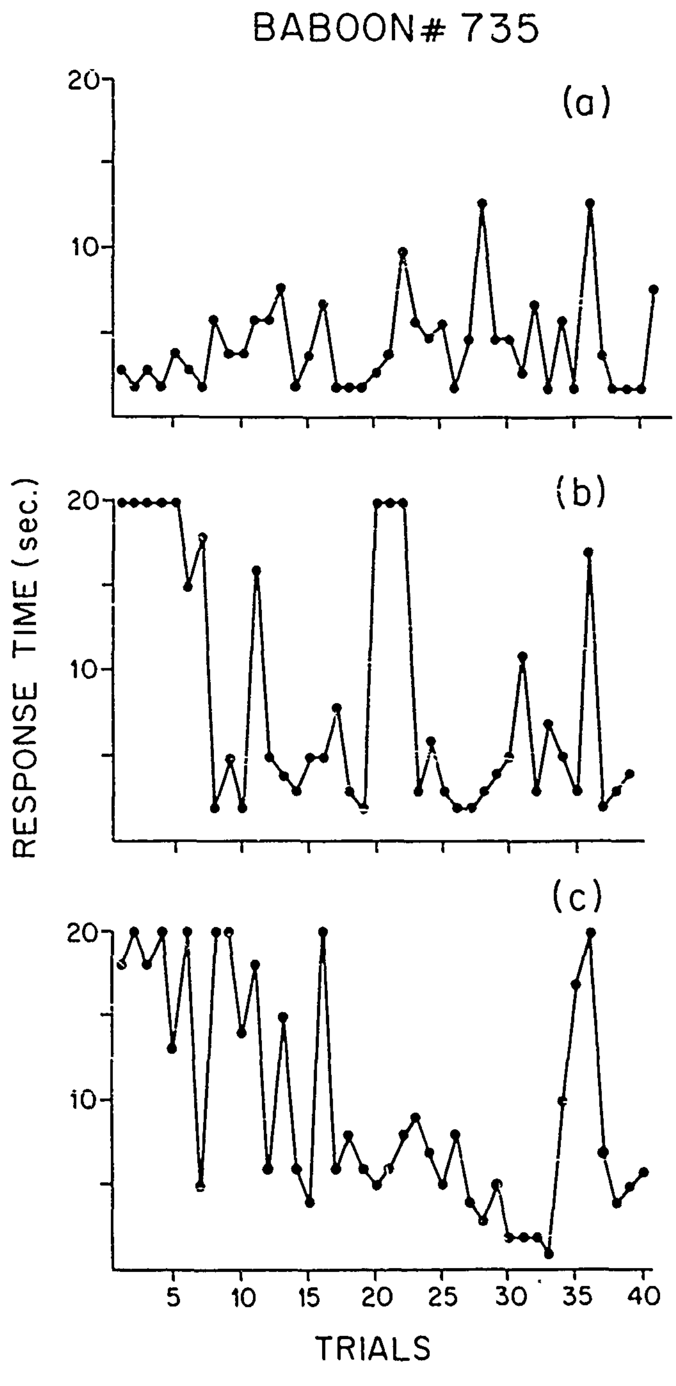

Figure 5. Association of attentional lapses to the discrimination task with a documented seizure for Baboon 735. (a) Trial-by-trial response time pattern on the day before this seizure (2 days after prior seizure); (b) Response time pattern for behavioral session initiated within 45 min after animal recovered from generalized seizure without treatment; (c) Response time pattern for session on day following the seizure.

CONCLUSIONS

Repeated, sublethal soman produces a characteristic profile of effects upon discrimination behavior in the baboon. This behavioral profile encompasses immediate, persistent, and delayed effects.

Immediate, Dose-Related	Persistent	Delayed
Attentional Deficits Increased Mean Session Response Time Decreased Percentage Trials Worked	Attentional Deficits (Intermittent, random basis)	Sudden, marked increase in extra responses occurring weeks after exposure and persisting for 1 or more weeks (data not shown).
Increased Incidence of Errors	Possible Recurring Seizures	
Decreased Extra Responses		
Generalized Seizures/Convulsions (at doses $\geq 5\ \mu g/kg$ soman)		

After exposure to a behaviorally effective dose of soman, baboons exhibited attentional deficits to the operant task on a random, intermittent basis. These attentional deficits have persisted to date and may be associated with generalized seizures or focal brain seizure activity. Both attentional deficits and recorded seizures appear to be increasing with time. These deficits appear to be analogous to the mental confusion seen in humans exposed to organophosphate compounds (3) and to be consistent with the persistent changes in brain electrical activity related to OP exposure reported by other investigators (4).

Results of these studies indicate that sublethal soman exposure may produce CNS lesions which are not seriously incapacitating at the time of exposure, but which may worsen and impair task performance as well as become life threatening at some later time.

R E F E R E N C E S

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EFFECTS OF SOMAN ON ACQUISITION OF DISCRIMINATED AVOIDANCE-ESCAPE BEHAVIOR BY RATS

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Groups of male Sprague-Dawley rats were injected subcutaneously with either saline, 31 μg/kg soman or 46 μg/kg soman. Injections, spaced 3 days apart, were given twice prior to beginning avoidance behavior training and twice during training week 1. Blood samples obtained 24 hr prior to the first soman administration and 24 hr after the last soman administration were analyzed for plasma and whole blood AChE activity; inhibition of plasma or whole blood AChE was dose related. The avoidance-escape task required that the rats learn to press a lever in the presence of a tone stimulus in order to avoid a mild electric shock or to press the lever in the presence of the shock and thereby escape it. By training week 12, the avoidance behavior task was acquired by five of eight saline rats, by four of seven of the 31 μg/kg soman rats and by zero of seven of the 46 μg/kg soman rats. Since almost all of the rats that did not learn the avoidance response pressed the lever at the shock onset (escape responses), the inability to acquire the avoidance behavior is not due to a soman-induced motor impairment. These findings indicate that subchronic administration of soman at a dose of 46 μg/kg (or .75 x LD_{50}) produces behavioral deficits as reflected in an inability to learn an auditory discriminated avoidance task. Histological evaluation of the brains of these animals is in progress. Attempts will be made to correlate behavioral findings with brain pathology.

This work supported in part by the U.S. Army Medical Research and Development Command under Contract DAMD-17-82-C-2161.

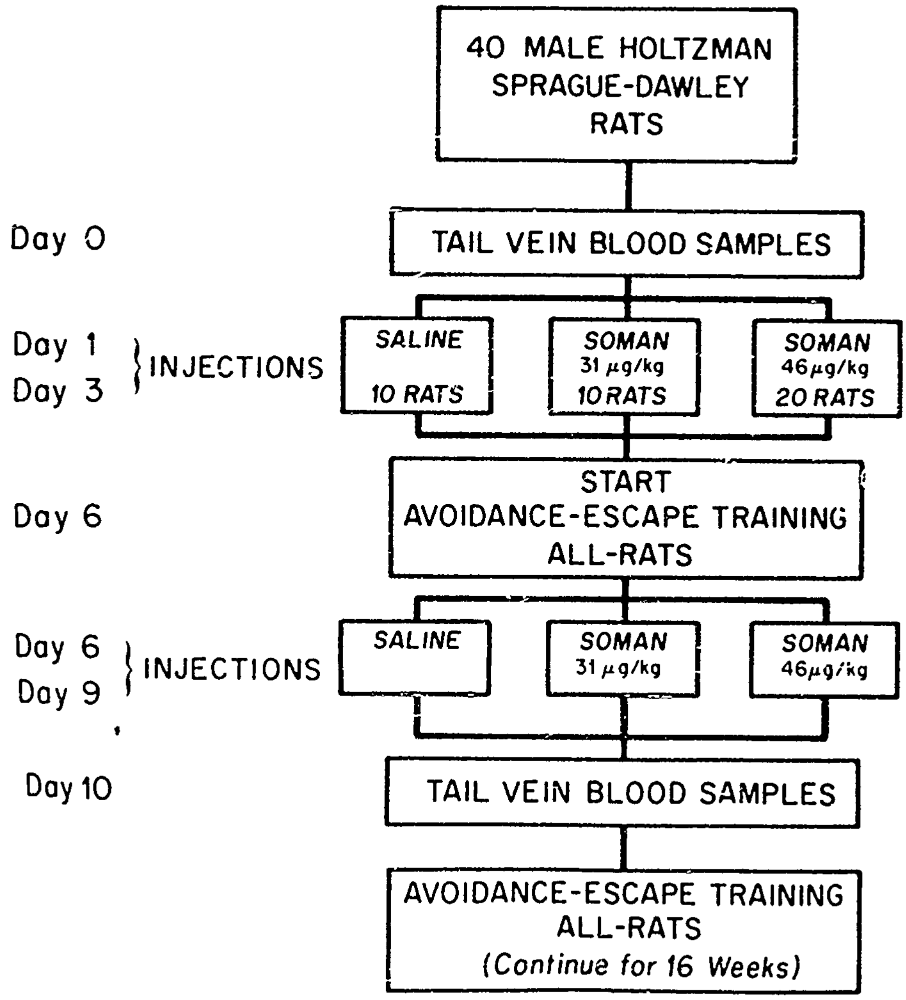
INTRODUCTION

Ongoing research in our laboratories has been concerned with the effects of subchronic administration of soman on performance of operant behavior by laboratory rats. For animals trained to perform these operant tasks, administration of soman at 5, 10, 20, or 40 (8-70% LD_{50}) μg/kg once every 3 days during a 30-day period yielded unsystematic data which generally reflected a reduction in operant rates during the injection periods with a return to baseline control levels after cessation of soman for those animals that survived the injections.

There have been several reports indicating persistent behavioral changes in survivors of near-lethal doses of soman (Mays et al., 1984; McDonough, Smith and Smith, personal communication, 1984). The latter investigators reported that after a single near-lethal dose of soman, rats were unable to learn to perform on a DRL schedule as well as control animals. The effect persisted up to 70 days post drug when the experiment was discontinued. These studies used acquisition or learning of a task rather than performance as the dependent variable.

The purpose of the present investigation was to determine if rats could learn a discriminated avoidance escape task after receiving several administrations of soman.

METHOD



RESULTS

The dosing protocol employed for the avoidance animals produced a lowering of blood AChE activity; the effects are shown in Table 1 for each treatment group. While the whole blood AChE level of the saline control group declined approximately 5% over the 10-day interval of soman administration, the lower soman dose group (.5 X LD_{50}) exhibited a 20% inhibition and the higher dose group (.75 X LD_{50}) exhibited a 24% inhibition 24 hr after the fourth soman injection. Plasma AChE activity was stimulated 21% by the saline injections, but was inhibited to the extents of 17 and 25%, respectively, by the soman injections.

TABLE 1. EFFECT OF SUBCHRONIC SOMAN UPON BLOOD AChE LEVELS OF DISCRIMINATED AVOIDANCE RATS

			AChE Activity			
			Whole Blood		Plasma	
Group	(N)	Time	μmol/hr/ml	% Inhibition	μmol/hr/ml	% Inhibition
Saline		Pre-	45.9 ± 1.7	0	17.4 ± 1.8	0
Control	(8)	Post-	43.7 ± 2.9	4.8	21.0 ± 2.1	-20.7
31 μg/kg		Pre-	42.5 ± 1.3	0	15.7 ± 0.9	0
(.5 LD_{50})	(7)	Post-	33.9 ± 2.6	20.2	13.0 ± 1.2	17.2
46 μg/kg		Pre-	41.8 ± 1.9	0	19.5 ± 1.9	0
(.75 LD_{50})	(7)	Post-	31.8 ± 1.4	23.9	14.6 ± 1.6	25.1

Soman or saline as indicated were administered four times over 10 days; blood samples taken 24 hr before first dose (pre-) and 24 hr after fourth dose (post-).

Values shown are group means ± S.E.M.

Figures 1, 2 and 3 contain avoidance and escape data obtained for each individual rat for each treatment condition. The ordinate indicates percent avoidance efficiency (X——X) plus S.E.M. and percent escape efficiency (•——•) minus S.E.M. The efficiency scores were calculated as percentage of responses to stimuli/total stimuli (avoidance) or percentage of responses to shocks/total shocks (escape).

Figure 1 shows the effects of subchronic saline injections on acquisition of the avoidance and escape behavior. Escape responding efficiency reached 100% for all saline rats. Five of the eight rats learned to avoid with efficiency scores of better than 50%; avoidance efficiency reached 95% for rats A-1 and A-19 and ranged from 50 to 80% for the other three rats that learned the avoidance response. Although rats A-16, A-22 and A-28 did make avoidance responses, their efficiency remained below 40%.

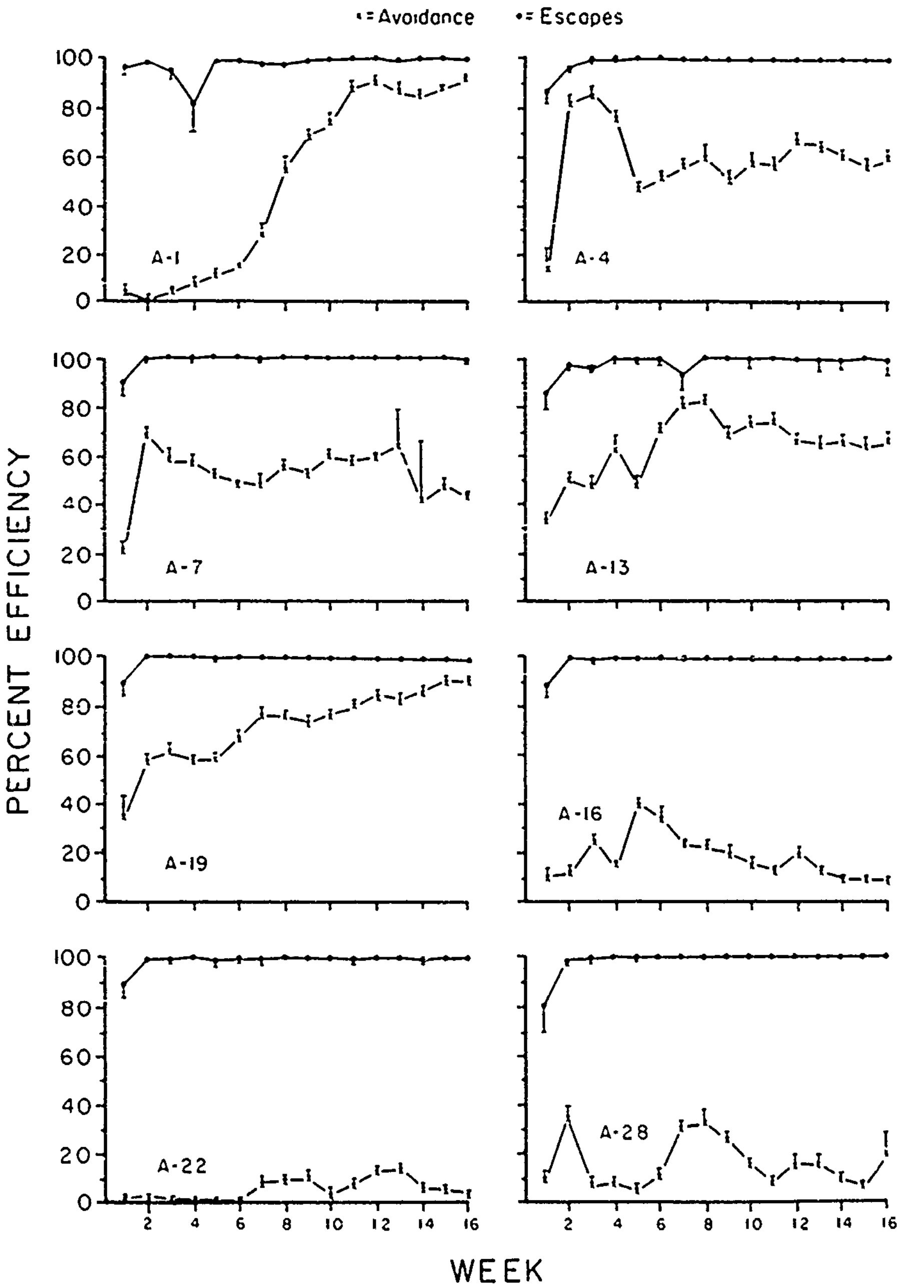


Fig. 1. Effects of subchronic saline administration on acquisition of a lever-pressing avoidance and escape response by laboratory rats. Avoidance efficiency = responses during stimuli/total stimuli x 100 and escape efficiency = responses during shocks/total shocks x 100.

Figure 2 shows the effects of subchronic soman at 31 $\mu g/kg$ (.5 X LD_{50}) on acquisition of the avoidance-escape behavior. Escape responding efficiency reached 100% for all rats. By the ninth week of training, escape responses for rat A-17 began to diminish, reaching zero level by week 16. Direct observation of the rat revealed it to be lying on its back and avoiding shocks by insulating itself with its fur. Four of the 31 $\mu g/kg$ rats acquired the lever-pressing avoidance response. Rats A-5 and A-8 stabilized at better than 60% efficiency while avoidance efficiency for rats A-11 and A-26 ranged from 25 to 75%. Two of the rats in this group made no avoidance responses during the period of 16 weeks.

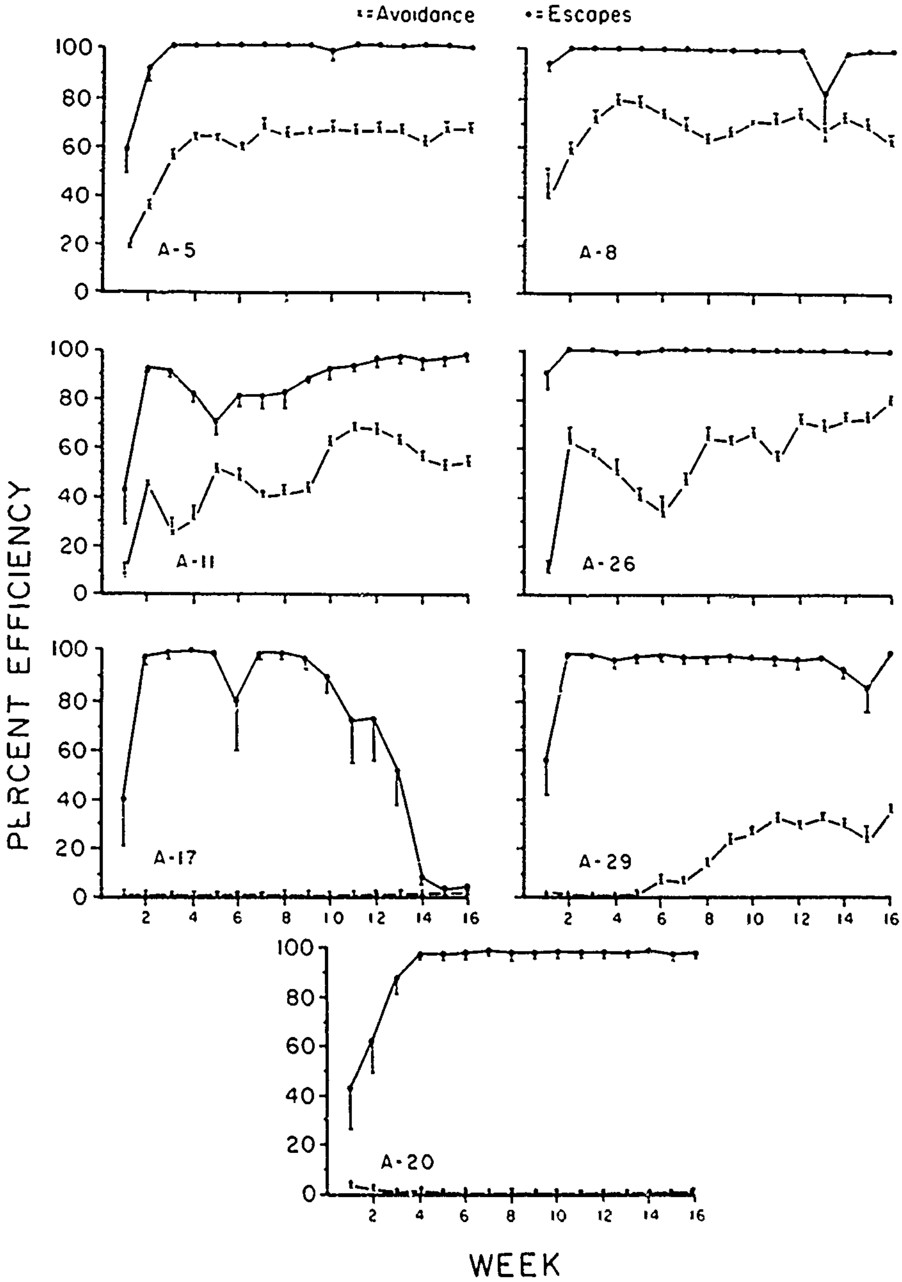


Fig. 2. Effects of subchronic administration of 31 $\mu g/kg$ soman on acquisition of a lever-pressing avoidance and escape response by laboratory rats. Avoidance efficiency = responses during stimuli/total stimuli x 100 and escape efficiency = responses during shocks/total shocks x 100.

Figure 3 shows the effects of subchronic soman at 46 μg/kg (.75 X LD_{50}) on acquisition of avoidance-escape behavior. None of the 46 μg/kg soman animals acquired the avoidance response during a 16-week training period. Escape responding efficiency reached 100% for five rats in this group. Periodic observation of rats A-33 and A-36 showed them to be standing and taking the shocks, during the early part of the study, and consistently lying on their backs throughout the sessions beginning the 11th week of the study. It appeared as though rat A-3 might learn to avoid but this rat reached a maximum efficiency of 35% during week 4 and then became progressively worse, reaching a low of 12% during week 16.

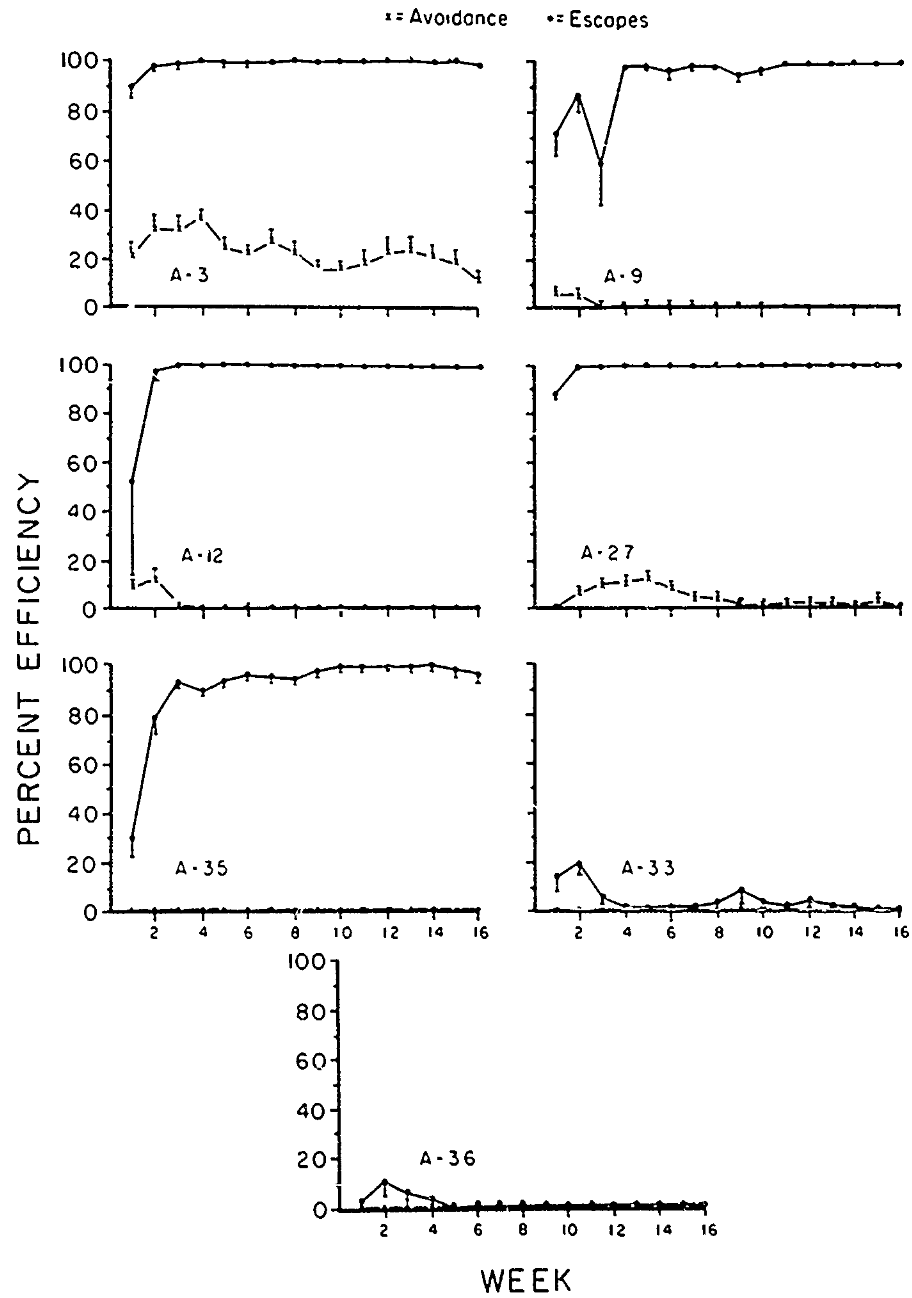


Fig. 3. Effects of subchronic administration of 46 μg/kg soman on acquisition of a lever-pressing avoidance and escape response by laboratory rats. Avoidance efficiency = responses during stimuli/total stimuli x 100 and escape efficiency = responses during shocks/total shocks x 100.

DATA ANALYSIS

Analysis of variance with repeated measures revealed significant dose effects. Acquisition data of the saline or 31 μg/kg soman rats were significantly different from the data of the 46 μg/kg soman rats. The data of the saline rats didn't differ significantly from those of the 31 μg/kg soman animals. There was a significant dose X week interaction indicating that avoidance behavior improved over time for the saline and 31 μg/kg but not for the 46 μg/kg group. Analysis of variance with repeated measures for the learners of the saline and 31 μg/kg groups revealed no significant dose effects in avoidance acquisition but there was a significant dose X week interaction indicating an improvement in avoidance behavior over time for both groups.

Animals were sacrificed and brains subjected to histological examination. Tissues were perfusion-fixed with Karnowski's fixative. At this time, no overt neuronal necrosis has been recognized in sections examined with light microscopy, although there does seem to be an increase in the number of dark shrunken neurons in the treatment groups versus the control group. Currently, a study is being conducted to quantify the neuronal changes which are most evident in the pyramidal layer of the hippocampus. Electron microscopic examination of select CNS sites is planned.

CONCLUSIONS

1. Rats given 46 μg/kg soman every 3 days for a total of four injections were unable to learn a discriminated avoidance response after 80 training sessions. Inability to acquire the avoidance response cannot be attributed to a soman-induced motor impairment since most of the rats were able to escape shock.

2. This observation, coupled with our previous findings that subchronic administration of soman produced no systematic or persistent effects on performance of a task already learned, suggests that subchronic soman may inhibit the learning of new behaviors but does not produce lasting effects on established behaviors.

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OPERANT BEHAVIOR OF NONHUMAN PRIMATES SURVIVING EXPOSURE TO TWICE THE LD50 OF SOMAN

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OBJECTIVE

USE PERFORMANCE TO ASSESS FUNCTIONAL CAPABILITY FOLLOWING SOMAN EXPOSURE WITH SUPPORTIVE THERAPY.

EXPOSURE

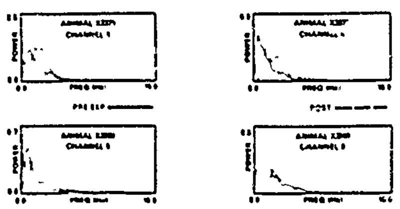
- PENTOBARBITAL
- INTUBATE
- IV (13 μg/kg)
- ATROPINE
- VENTILATE (HFJ)
- 24-HOUR TEST

RESULTS

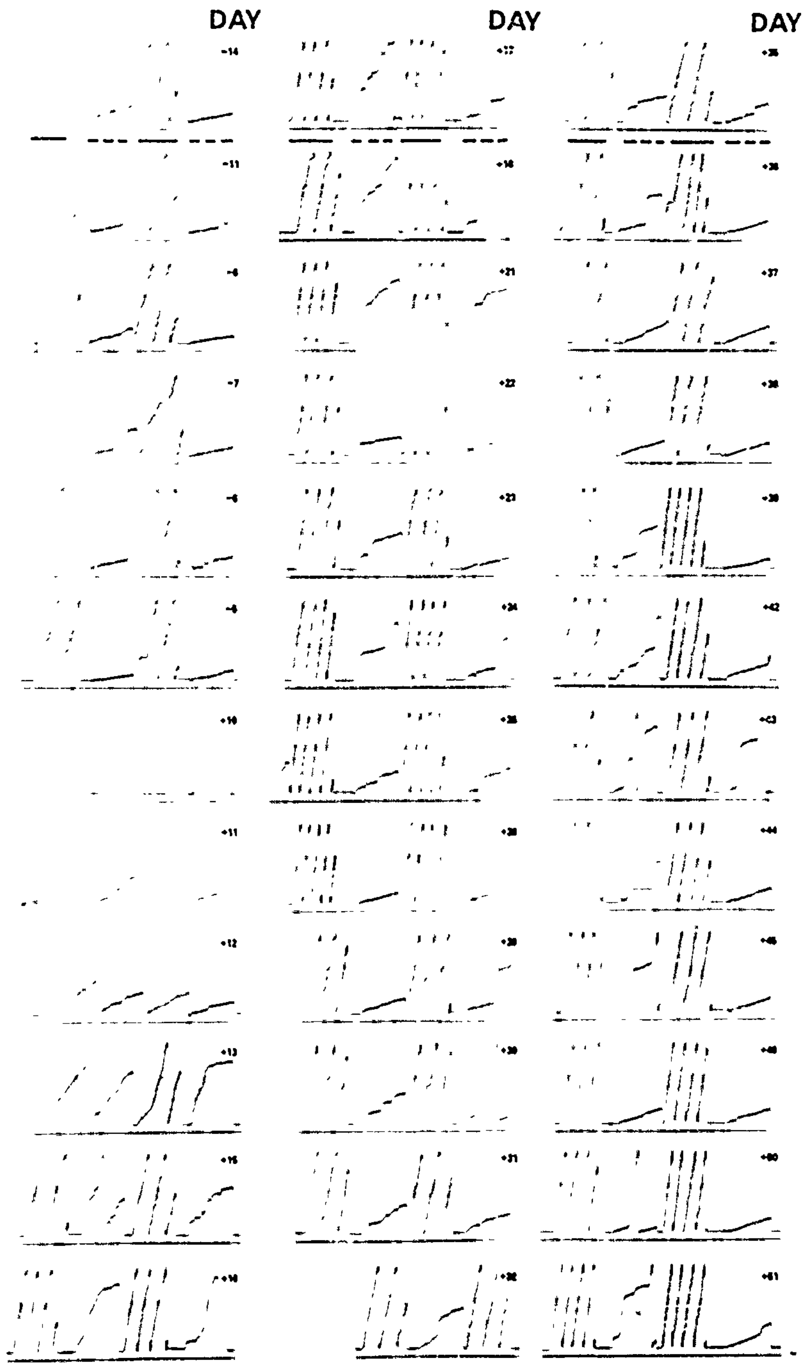
SIX WEEKS AFTER EXPOSURE, APPARENTLY NORMAL BABOONS HAD ALTERED FR/DRL RESPONSE PATTERNS.

CONCLUSION

SEEMINGLY HEALTHY SURVIVORS MIGHT SHOW BEHAVIORAL DEFICITS WHICH LIMIT ABILITY TO FUNCTION.



FOUR CHANNELS OF PRE- AND POST-EXPOSURE EEG DATA, COLLECTED WITH SUBJECTS UNDER CHLORALOSE ANESTHESIA, WERE EXAMINED BY POWER SPECTRUM ANALYSIS. THE PLOTS SUGGEST AN INCREASE IN LOW FREQUENCY BRAIN ACTIVITY, BUT DIFFERENT LEVELS OF ANESTHESIA CANNOT BE DISCOUNTED.



FR —— DRL - - -

BABOON 2371

OPERANT BEHAVIOR OF NONHUMAN PRIMATES SURVIVING EXPOSURE TO TWICE THE LD50 OF SOMAN

J. L. ORR and W. R. ROGERS C. D. WHITE and G. T. MOORE
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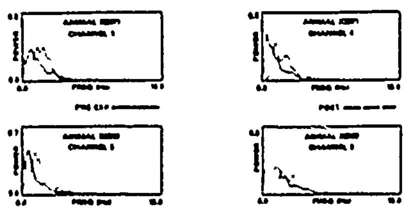

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FR —— DRL - - -

BABOON 2599

PERFORMANCE ON AN ALTERNATION TASK FOLLOWING ACUTE EXPOSURE TO SOMAN

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ABSTRACT

Following preliminary training to lever press for milk reinforcement, seventy adult male rats were divided into four groups and injected sc with saline, 75, 85 or 95 ug/kg soman. After recovery, surviving rats were retrained to lever press. When an animal obtained 100 reinforcements within a 30 minute period, alternation training began. During alternation training the correct lever was indicated by a cue light. Responses on the uncued lever were counted as incorrect responses and not reinforced. As animals became proficient at the task the requirements were gradually raised until the terminal performance level of Fixed Ratio 20 (FR20) with a 20 sec intertrial interval (ITI20) was reached. Training sessions of 40 min per day, 5 days per week were continued until the animals attained a criterion of less than 25% incorrect responses for 3 consecutive days or 100 training sessions had been given.

Soman produced lethality and significant increases in acute behavioral toxicity as a function of dose. Although all saline animals and nine out of ten animals given 75ug/kg managed to perform the alternation at FR20/ITI20; only approximately a third of the animals given either 85 or 95 ug/kg managed to perform at this level. When criterion performance is considered, the results were similar in that all saline animals reached criterion while only 60%, 33% and 33% of the 75, 85 and 95 ug/kg groups reached criterion. The mean number of days to attain FR20/20ITI and to reach criterion produced similar dose dependent results. The results of pathology confirmed previous findings. Both 85 and 95 ug/kg soman produced severe pathology with cortical atrophy and ventricular dilation. Severe neural depletion was also seen in the hippocampus, amygdala and thalamic nuclei. These data confirm previous results of long-term learning and performance deficits in soman exposed rats and suggest that the observed deficits may be due to the damage produced by soman in the limbic system.

PURPOSE

ARE LONG-TERM BEHAVIORAL DEFICITS RELATED TO NEURAL PATHOLOGY

Animals exposed to convulsion producing doses of the toxic organophosphate, soman, exhibit neural damage in a number of limbic sites, including the hippocampus and septal nucleus (McLeod et al, 1984). Rats surviving these soman-induced convulsions will also demonstrate numerous behavioral deficits including increased reactivity to tactile stimulation and deficits in the acquisition of a DRL task (Smith et al, 1984). These behavioral abnormalities are similar to those seen in rats after experimentally produced lesions of the hippocampus and/or the septal nucleus (Grey and McNaughton, 1983). The purpose of this study was to determine whether the neural damage observed after soman exposure would produce decrements in the acquisition of a cued alternation task similar to those seen after experimentally produced limbic lesions in rats and whether these changes would be dose dependent.

METHOD

Seventy adult male rats were trained to lever press for milk reinforcement on a fixed ratio 1 (FR1) schedule in a two lever operant chamber. During this initial training, a response on either lever resulted in reinforcement. Following this training, the rats were divided into four groups and injected sc with either saline (10 rats), 75 ug/kg (14 rats), 85 ug/kg (21 rats) or 95 ug/kg soman (25 rats). One hour after injection, all animals were rated for acute behavioral toxicity using the scale developed by Penetar et al. (1982). After a two week recovery period or reattainment of preinjection body weight, surviving animals were retrained to lever press. When an animal received 100 reinforcements on either lever during a 30 minute period, alternation training began. During alternation training, the correct lever was indicated by illumination of the cue light over the correct lever. Responses on the uncued lever on any trial were counted as incorrect and not reinforced. Initially rats were required to simply alternate between the two levers using a FR1 schedule with a 2 sec intertrial interval (ITI2). As animals became proficient, requirements were gradually raised to the terminal level of FR20 with a ITI20. Training sessions were 40 min per day, 5 days per week. Training was conducted until rats reached criterion performance level of no more than 25% incorrect responses for three consecutive days or post-exposure training had been conducted for 100 days, whichever came first.

Following the conclusion of behavioral training all rats were injected with a lethal dose of pentobarbital and perfused with formalin solution. The brains were removed, sectioned and stained for light microscopy. A trained veterinary pathologist then examined each brain and rated it between 0 (no visible pathology) and 4 (severe pathology observed throughout the brain.

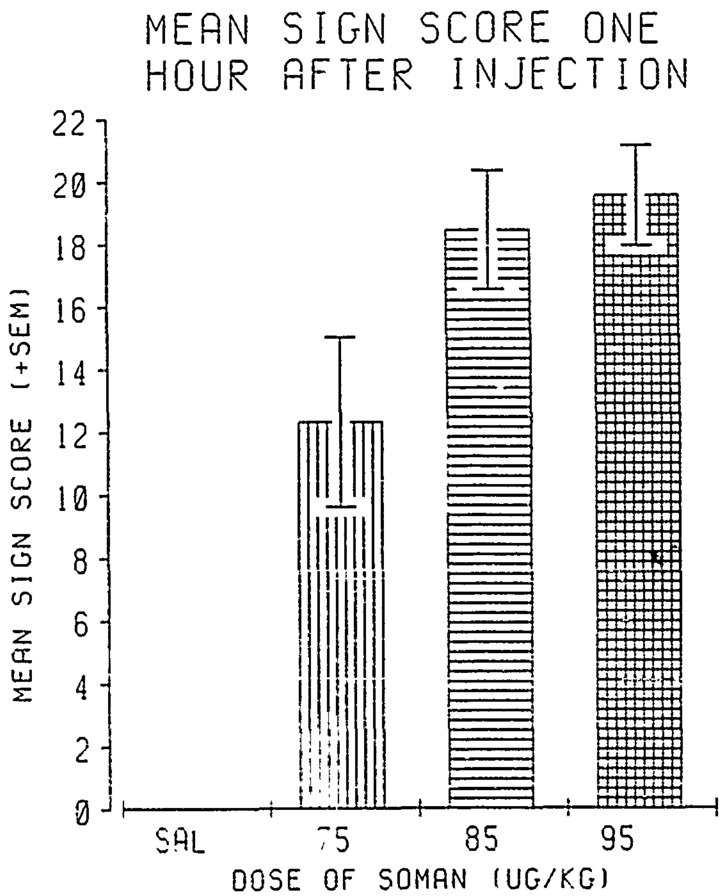


FIGURE 1

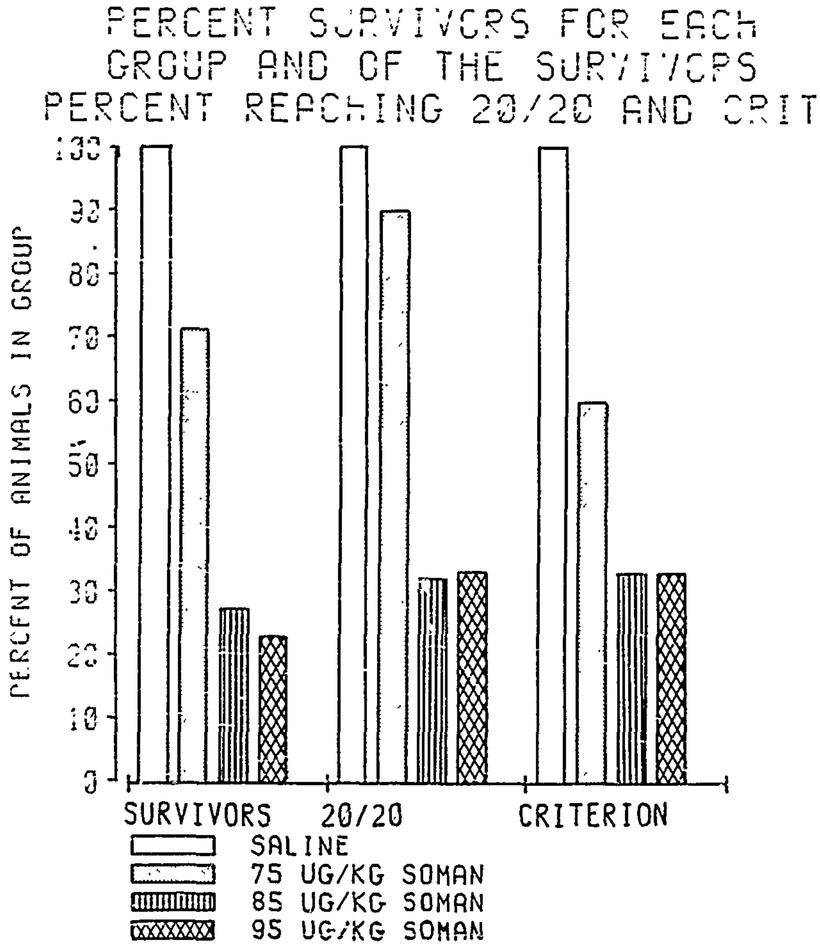


FIGURE 2

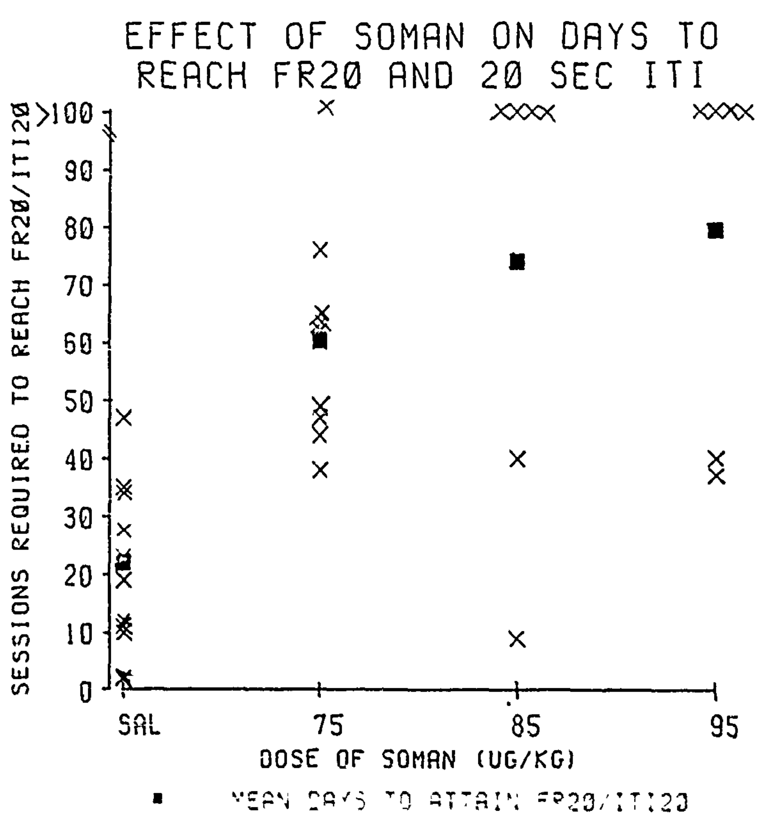


FIGURE 3

RESULTS

SIGNS AND LETHALITY

As may be seen in Fig 1, soman produced a significant dose related increase in signs of anticholinesterase intoxication one hour after injection ($F[3,66] = 15.4$, $p < .001$). Post-hoc analyses revealed that 75 ug/kg produced significantly fewer signs than 85 or 95 ug/kg ($p < .05$). The behavioral signs were positively related to the incidence of one week lethality seen in Fig 2. Once again, 75 ug/kg was significantly less lethal than 85 or 95 ug/kg soman.

ALTERNATION TASK DEFICITS

Fig 2 and Fig 3 show the percentage of surviving animals who were capable of performing the FR20/ITI20 alternation and the mean number of days required to reach this level. In Fig 2, only animals receiving 85 and 95 ug/kg were significantly different from the saline group.The group receiving 75 ug/kg was not significantly different from either saline or the two higher doses. When the mean number of days to reach FR20/ITI20 is examined, all soman groups required significantly longer than the saline group ($F[3,28] = 6.85$, $p < .01$). Because of this result, it is not surprising that significant differences were also found in the number of days to criterion as seen in Fig 4 ($F[3,28] = 7.60$, $p < .001$).

PATHOLOGY

Soman-injected rats demonstrated a variety of neural pathology as may be seen in Figs 5, 6 and 7. Both the dorsal and ventral hippocampus, thalamic and amygdaloid nuclei and the pyriform cortex demonstrated a gradation of damage including mineralization, malacia, spongiosis, and depletion of neurons. In the more severe cases the pathology included cortical atrophy, dilation of the ventricles and gliosis. The degree of pathology was found to dose-dependent with 6 of 10 animals receiving 75 ug/kg demonstrating none to minimal neural pathology (Fig 8). Only two animals in each of the 85 and 95 ug/kg groups showed low levels of pathology. All others demonstrated moderate to severe pathology. Significant correlations between the severity of neural pathology and both the number of days to FR20/ITI20 ($r = .80$) and days to criterion ($r = .67$) were seen across all soman doses.

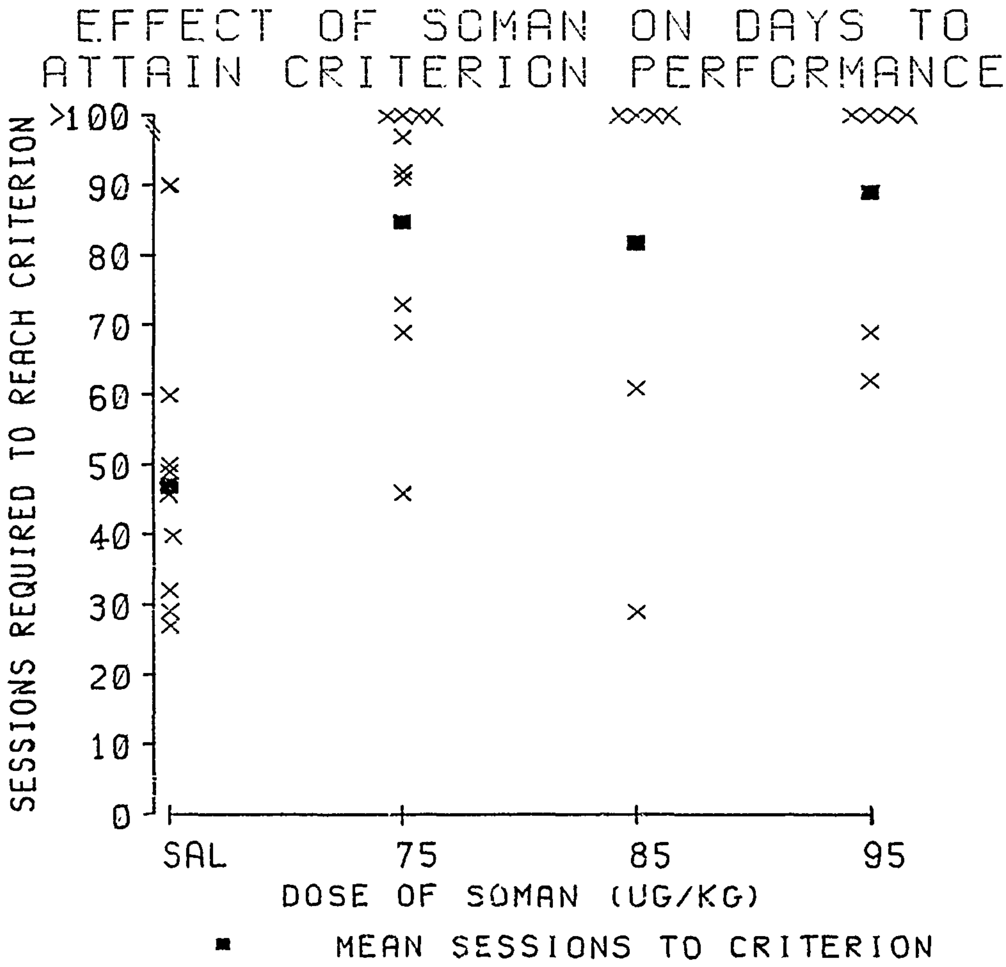

EFFECT OF SOMAN ON DAYS TO
ATTAIN CRITERION PERFORMANCE
SESSIONS REQUIRED TO REACH CRITERION
>100
90
80
70
60
50
40
30
20
10
0
SAL
75
85
95
DOSE OF SOMAN (UG/KG)
MEAN SESSIONS TO CRITERION

FIGURE 4

FIGURE 5. Section of the rat brain at the level of the third ventricle and lateral habenula. This illustrates cellular depletion in the dorsal hippocampus (upper right). Also shown are diffuse malacia and mineralization. The thalamic nuclei (lower right) demonstrates widespread spongiform changes.

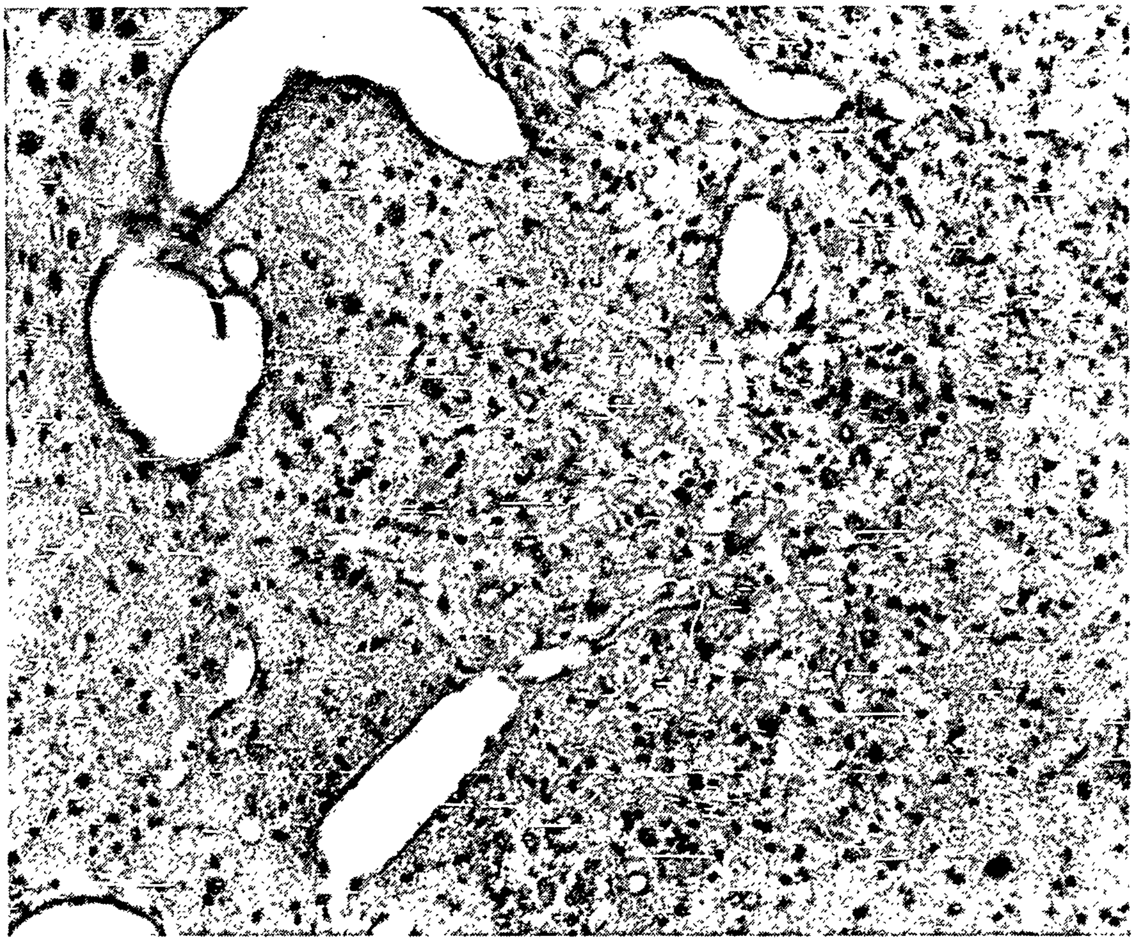


Figure 6. Severe and diffuse malacia of the lateral thalamic nuclei.

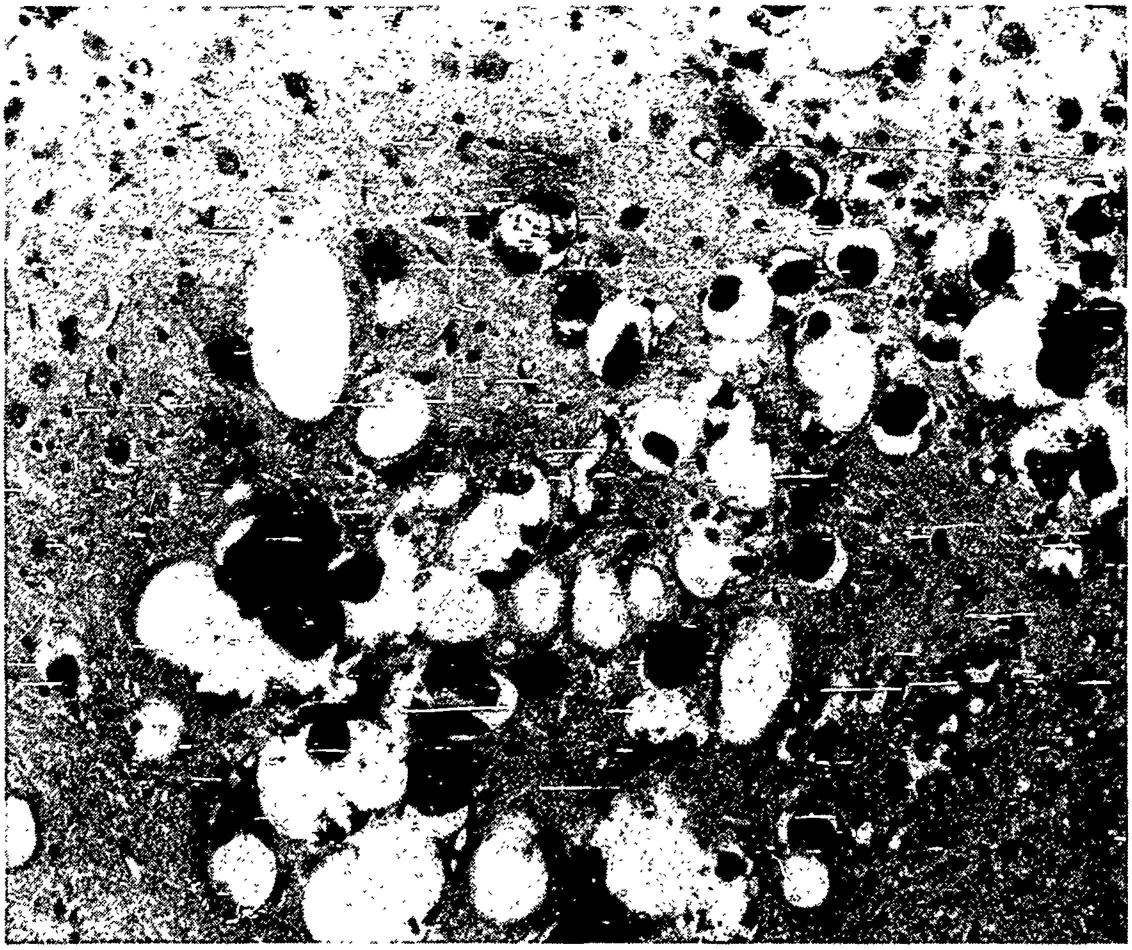
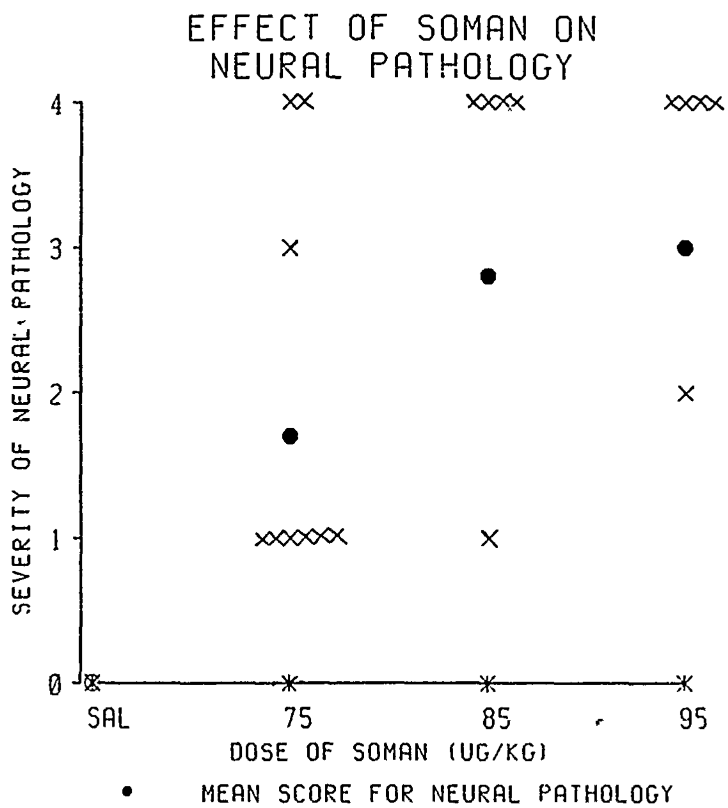


Figure 7. Neuronal necrosis, mineralization and malacia of the lateral thalamic nucleus.



EFFECT OF SOMAN ON
NEURAL PATHOLOGY
SEVERITY OF NEURAL PATHOLOGY
4
3
2
1
0
SAL
75
85
95
DOSE OF SOMAN (UG/KG)
MEAN SCORE FOR NEURAL PATHOLOGY

FIGURE 8

DISCUSSION

Although soman did produce a dose-related increase in both toxic signs and lethality, no significant dose-related differences were found in the days required to reach FR20/ITI20 or criterion. However when the severity of pathology is compared to the two measures of learning, a definite relationship is found. At all doses of soman, only those animals exhibiting no or minimal pathology performed better than the mean for each dose group. All animals exhibiting severe pathology were unable to reach criterion. That is, two populations were observed; those demonstrating minimal or no pathology and those with moderate and severe pathology. Animals of each type are found in every dose group, however the proportion of each type varies as a function of dose. At low doses of soman a high proportion of animals will show mild pathology and be able to learn the task though at a retarded rate. At higher doses more animals will demonstrate severe pathology and thus be unable to learn the task. The group receiving 75 ug/kg soman demonstrated a variety of behaviors. Most of these animals displayed mild behavioral toxicity and pathology and were able to both perform the FR20/ITI20 and attain criterion performance. Those who were unable to attain criterion also demonstrated severe neural pathology. Thus, we conclude that although soman does produce a decrement in the ability to learn a cued alternation task, this decrement is related to the severity of the pathology seen in the affected animals. Animals receiving a moderate dose of soman will tend to demonstrate a decrement in the acquisition of the task and show moderate pathology. Those animals who recover from >LD50 of soman will be unable to learn a cued alternation task and will demonstrate severe neural pathology.

CONCLUSIONS

1. Soman produced significant dose-related increases in toxic signs and lethality. There were also dose-related changes in the number of animals capable of both learning the task and attaining criterion.

2. The size of the correlations between the severity of brain pathology and learning decrements indicate a strong relationship between soman induced neural damage and the observed behavioral deficits.

3. These deficits are similar to those seen after experimental lesions of the hippocampus and amygdala. Therefore the conclusion may be made that the soman-induced lesions of the ventral hippocampus and amygdala were the basis of these deficits.

THE PERFORMANCE EFFECTS OF SOMAN EXPOSURE FOLLOWING 8 HOURS OF THERMAL STRESS IN RATS

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INTRODUCTION

THE INFLUENCE OF THERMAL STRESS ON THE PHYSIOLOGICAL MECHANISMS THAT MODIFY SUSCEPTIBILITY TO CHEMICAL AGENTS HAS ONLY RARELY BEEN INVESTIGATED. IN PARTICULAR, NO SYSTEMATIC STUDIES HAVE BEEN PERFORMED ON SOMAN TOXICITY AS A FUNCTION OF PRIOR THERMAL STRESS. THE STUDY REPORTED HERE EVALUATES THE EFFECTS OF SOMAN ON A NUMBER OF BEHAVIORAL TASKS AFTER AN 8-HR EXPOSURE TO ONE OF FIVE THERMAL STRESS CONDITIONS.

METHODS

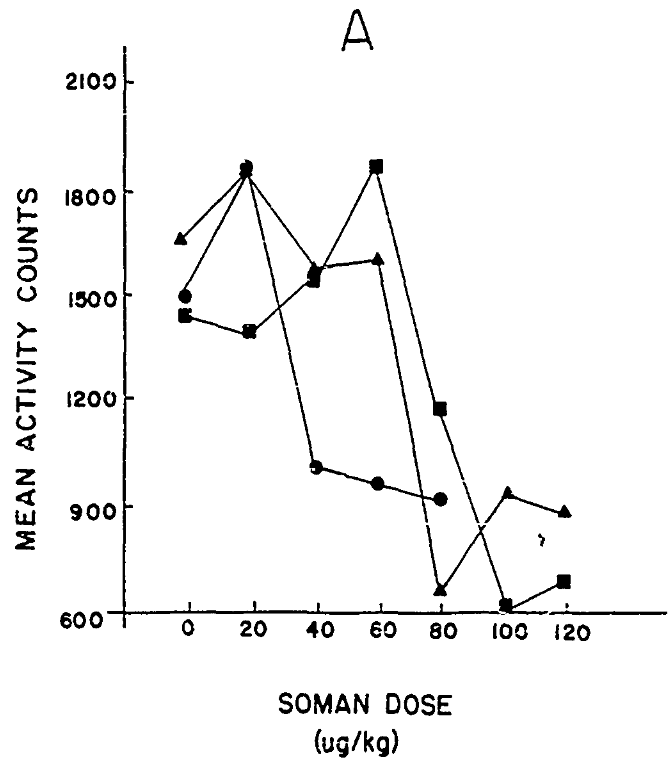
RATS WERE EXPOSED TO -1, 7, 15, 23, OR 31^{0}C (ALL AT 80 $\pm$ 5% RELATIVE HUMIDITY) FOR 8 HRS, REMOVED FROM THE ENVIRONMENTAL CHAMBER, INJECTED WITH SOMAN (0 TO 160 μG/KG DOSE RANGE), AND TESTED 30 MIN POSTINJECTION ON A SERIES OF TESTS. THE TEST BATTERY INCLUDED GENERAL MOTOR ACTIVITY, GRIP STRENGTH, CORE TEMPERATURE, SENSITIVITY TO HEAT (TAIL FLICK) AND ELECTRICAL SHOCK (PAW FLINCH), EFFECTS ON MEMORY (ONE-TRIAL PASSIVE AVOIDANCE), ABILITY TO LEARN A NEW TASK (SHUTTLE AVOIDANCE) AND A SUBJECTIVE RATING OF THE ANIMAL'S CONDITION. THE TEST BATTERY WAS ACCOMPLISHED IN THE ORDER LISTED.

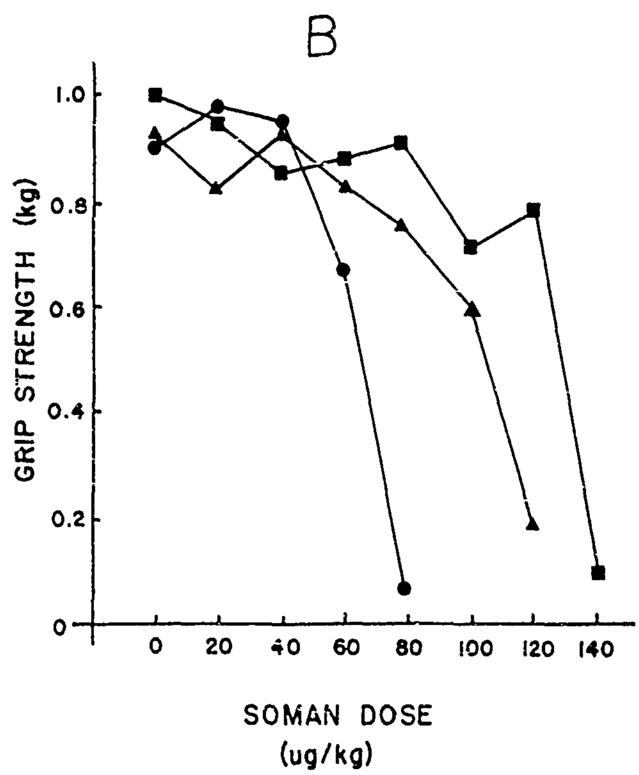
TABLE 1: EFFECTIVE DOSE (µG/KG) IN PRODUCING A 50% (ED50) PERFORMANCE DEFICIT AS AS FUNCTION OF THE PREEXPOSURE THERMAL STRESS TEMPERATURE.

STRESS TEMP°C	MOTOR ACT	GRIP	CORE TEMP	HEAT SENS	SHOCK SENS	ONE-TRIAL AVOID	SHUTTLE AVOID	SUB RATING	LD10
-1	38	63	44	20	68	58	-	69	76
7	49	60	64	60	51	52	46	64	83
15	71	100	68	72	74	67	62	77	105
23	70	81	76	78	69	79	60	87	92
31	82	126	67	89	86	86	60	97	122

RESULTS

IN GENERAL, THE LOWER THE STRESS TEMPERATURE, THE GREATER SUSCEPTIBILITY TO SOMAN (TABLE 1). THERMAL STRESS ALONE HAD A SIGNIFICANT EFFECT ON THERMAL SENSITIVITY, ONE-TRIAL PASSIVE AVOIDANCE, AND SHUTTLE AVOIDANCE PERFORMANCE WHILE SOMAN PRODUCED A SIGNIFICANT EFFECT ON ALL MEASURES. A SIGNIFICANT THERMAL STRESS/SOMAN INTERACTION WAS OBSERVED FOR ACTIVITY, THERMAL SENSITIVITY, AND SUBJECTIVE RATING. THE EFFECTS OF SOMAN EXPOSURE WERE DEPENDENT ON PRIOR THERMAL STRESS CONDITIONS. THIS WAS SEEN AS A SHIFT OF THE SOMAN DOSE-RESPONSE FUNCTIONS TO THE RIGHT FOR HIGHER STRESS TEMPERATURE CONDITIONS (FIG. 1). FOR EXAMPLE, THE ED50 FOR THE ACTIVITY MEASURE WAS 38 µG/KG FOR THE -1°C EXPOSURE GROUP AND 82 µG/KG FOR THE 31°C GROUP (TABLE 1).


A
MEAN ACTIVITY COUNTS
2100
1800
1500
1200
900
600
0
20
40
60
80
100
120
SOMAN DOSE
(ug/kg)


B
GRIP STRENGTH (kg)
1.0
0.8
0.6
0.4
0.2
0
0
20
40
60
80
100
120
140
SOMAN DOSE
(ug/kg)

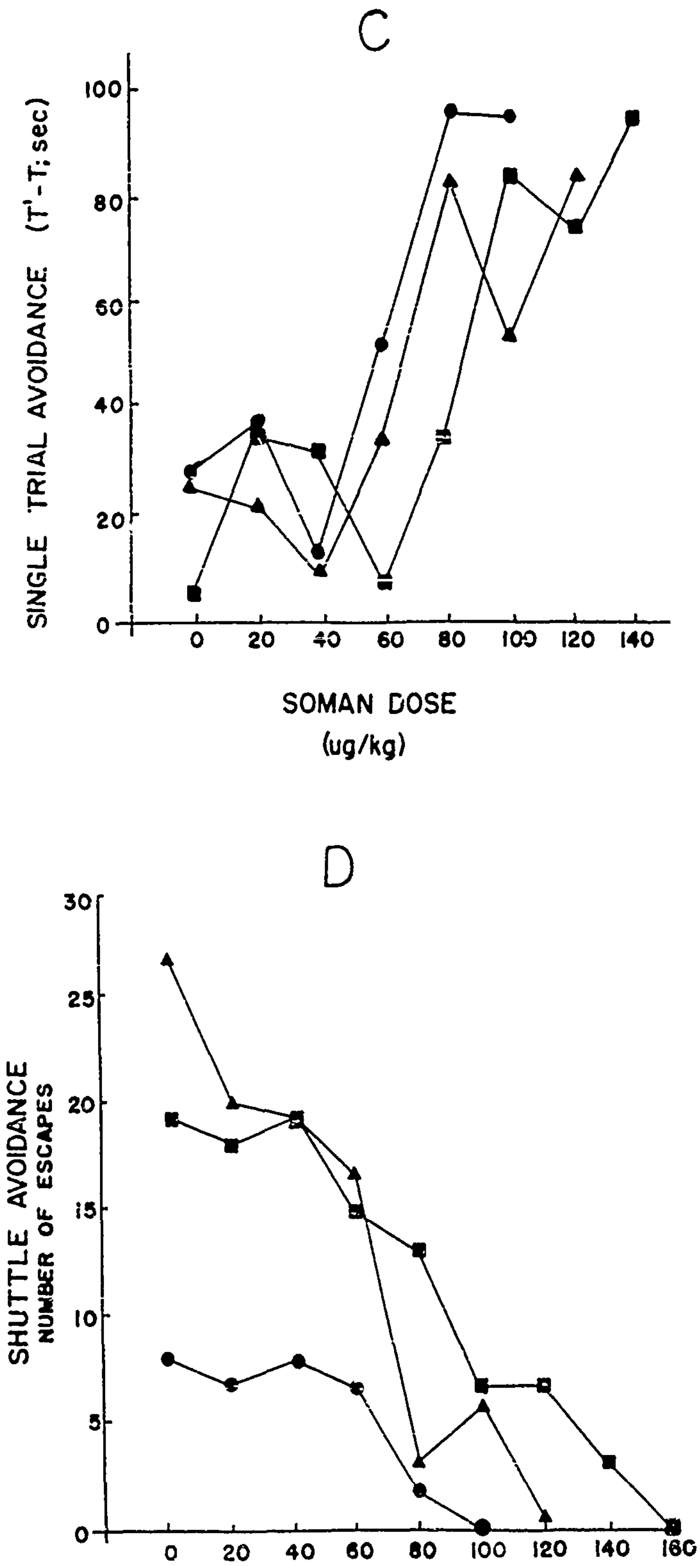


FIGURE 1 : PERFORMANCE RESULTS FOR FOUR TASKS AND THREE THERMAL STRESS CONDITIONS : ● -1°C; ▲15°C AND ■ 31°C.

DISCUSSION

THESE DATA SUGGEST THAT SOMAN TOXICITY WAS A FUNCTION OF THE EXTENT OF PREVIOUS METABOLIC ACTIVITY. IT IS NOT CLEAR WHETHER THE METABOLIC ACTIVITY WAS PRIMARILY SKELETAL MUSCLE ACTIVITY. IT WAS THE CASE FOR ALL PERFORMANCE TASKS THAT DEFICITS OCCURRED AT THE SOMAN DOSE THAT PRODUCED MOTOR FAILURE. STUDIES ARE NOW UNDERWAY TO DETERMINE IF SOMAN TOXICITY IS RELATED TO THE EXTENT OF PREVIOUS MOTOR ACTIVITY.

CORRELATIONS BETWEEN BRAIN REGIONAL CHOLINESTERASE ACTIVITY AND SIGNS OF SOMAN TOXICITY

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ABSTRACT

Soman is a potent organophosphate cholinesterase (ChE) inhibitor which reportedly depresses ChE activity in a dose-related manner in rat brain regions (Transact. Am. Soc. Neurochem., 14: 148, 1983). This relationship has been studied further in an attempt to correlate inhibition of brain regional ChE activity (BRChE) with observable signs of organophosphate toxicity at a variety of soman doses. Such correlation may prove useful in predicting BRChE in animal preparations where such activity cannot be measured directly, e.g., in brain tissue fixed by microwave irradiation. Rats were injected subcutaneously with a single dose of 0.3, 0.5, 0.6 or 0.7 LD_{50} of soman and were subsequently killed at selected time points. Animals were scored for toxic signs just prior to termination, according to the following "quality of life" (QL) categories: 0 = sign-free; 1 = ataxia, muscle fasciculations and/or licking/chewing behavior; 2 = salivation, limb weakness with hindlimb splaying, tremors, jerking and/or convulsions; and 3 = moribund with loss of righting reflex. Occasionally, intermediate categories (i.e. 0^+, 1^+, 2^+) were used. Toxic signs could usually be identified within 10 minutes after soman exposure. BRChE was assayed in brainstem (BST), cortex (CTX), hippocampus (HIP), midbrain (MB), cerebellum (CB) and striatum (STR) by the method of Groff et al (Clin. Toxicol., 9: 353, 1976) and is expressed as % of control activity ($\pm$ standard error). At 0.3 LD_{50} all animals (24/24) were free of signs of intoxication (QL = 0). Similarly, at 0.5 LD_{50}, virtually all (28/30) animals were sign-free. At 0.3 LD_{50} BRChE was minimally affected and showed $91 \pm 2\%$, $96 \pm 2\%$, $89 \pm 2\%$, $88 \pm 1\%$, $81 \pm 2\%$, and $103 \pm 5\%$ of control ChE activity, whereas at 0.5 LD_{50}, BRChE was moderately affected and showed $70 \pm 3\%$, $65 \pm 3\%$, $59 \pm 4\%$, $69 \pm 3\%$, $62 \pm 4\%$ and $91 \pm 2\%$ in BST, CTX, HIP, MB, CB and STR, respectively. However, at 0.6 and 0.7 LD_{50} recognizable signs of toxicity (QL = 1 or above) were apparent in 52% (43/82) and 91% (29/32) of the animals, respectively. Furthermore, a correlation between QL and BRChE existed in each brain region examined. The coefficients of correlation (R) were 0.86, 0.77, 0.78, 0.87, 0.83 and 0.94 at 0.6 LD_{50}, and 0.70, 0.67, 0.63, 0.69, 0.65 and 0.71 at 0.7 LD_{50} in BST, CTX, HIP, MB, CB and STR, respectively. Data for the 0.6 and 0.7 LD_{50} doses were combined and the R for QL vs. BRChE were 0.82 (BST), 0.72 (CTX), 0.74 (HIP), 0.81 (MB), 0.78 (CB) and 0.84 (STR). Thus, we have demonstrated that in rats acutely exposed to soman at doses greater than 0.5 LD_{50}, various signs of intoxication may be observed and, regardless of dose, the magnitude of inhibition of BRChE correlates well with the severity of these recognizable toxic signs.

INTRODUCTION

- Soman, a potent organophosphate cholinesterase (ChE) inhibitor, depresses ChE activity in a dose-related manner in rat brain regions (Shih, 1983).
- Soman produces a variety of toxic signs with variable degree of severity, reportedly related to the extent of brain ChE inhibition (Jovic, 1974).
- Study of brain cholinergic neurotransmitter function often requires head-focused microwave fixation, thus precluding direct, concurrent measurement of brain regional ChE activity.

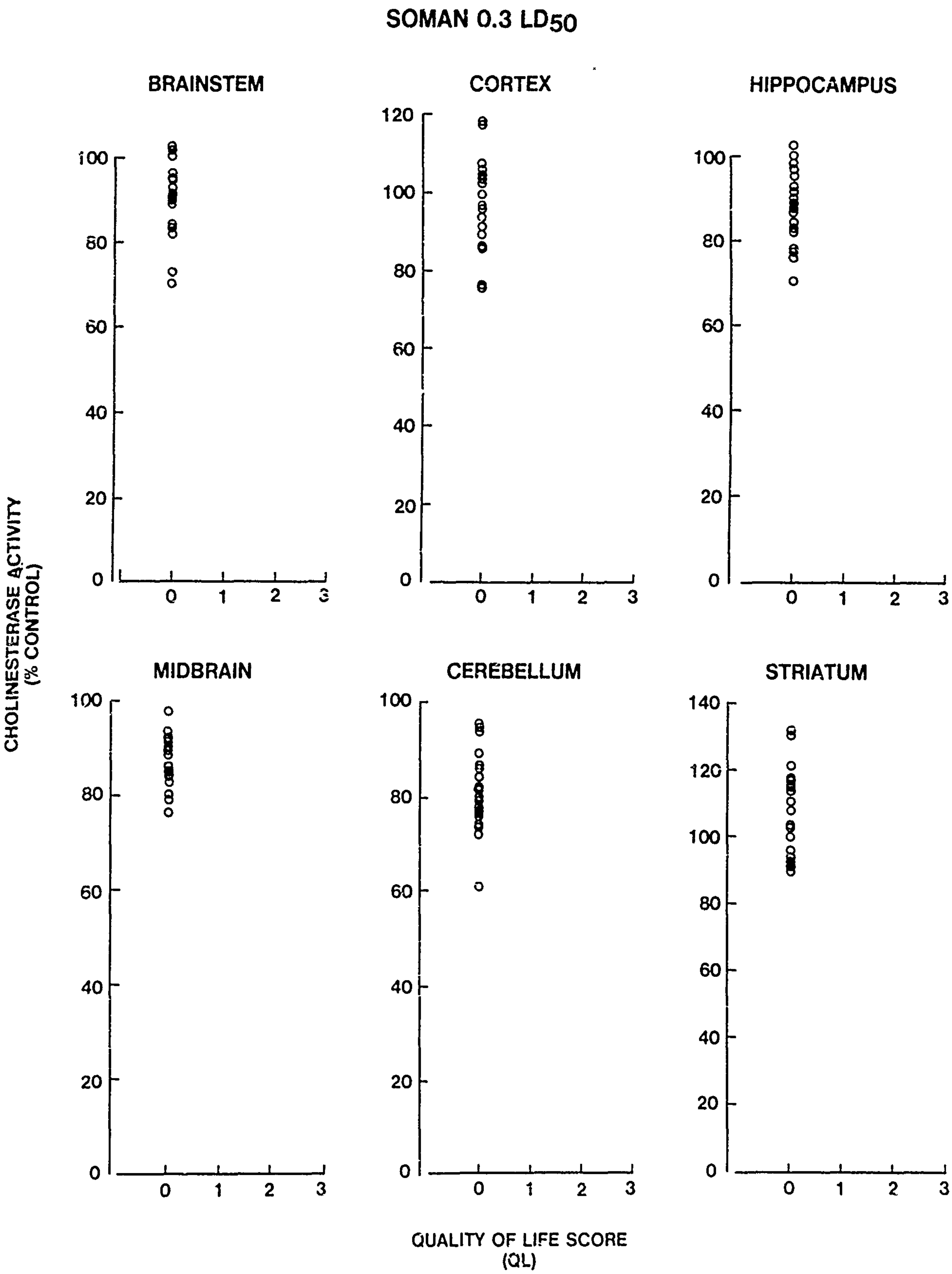
PURPOSE

- To correlate signs of organophosphate toxicity with brain regional ChE activity at several soman doses. Such a correlation may allow prediction of brain regional ChE activity by evaluating the severity of toxic signs.

MATERIALS AND METHODS

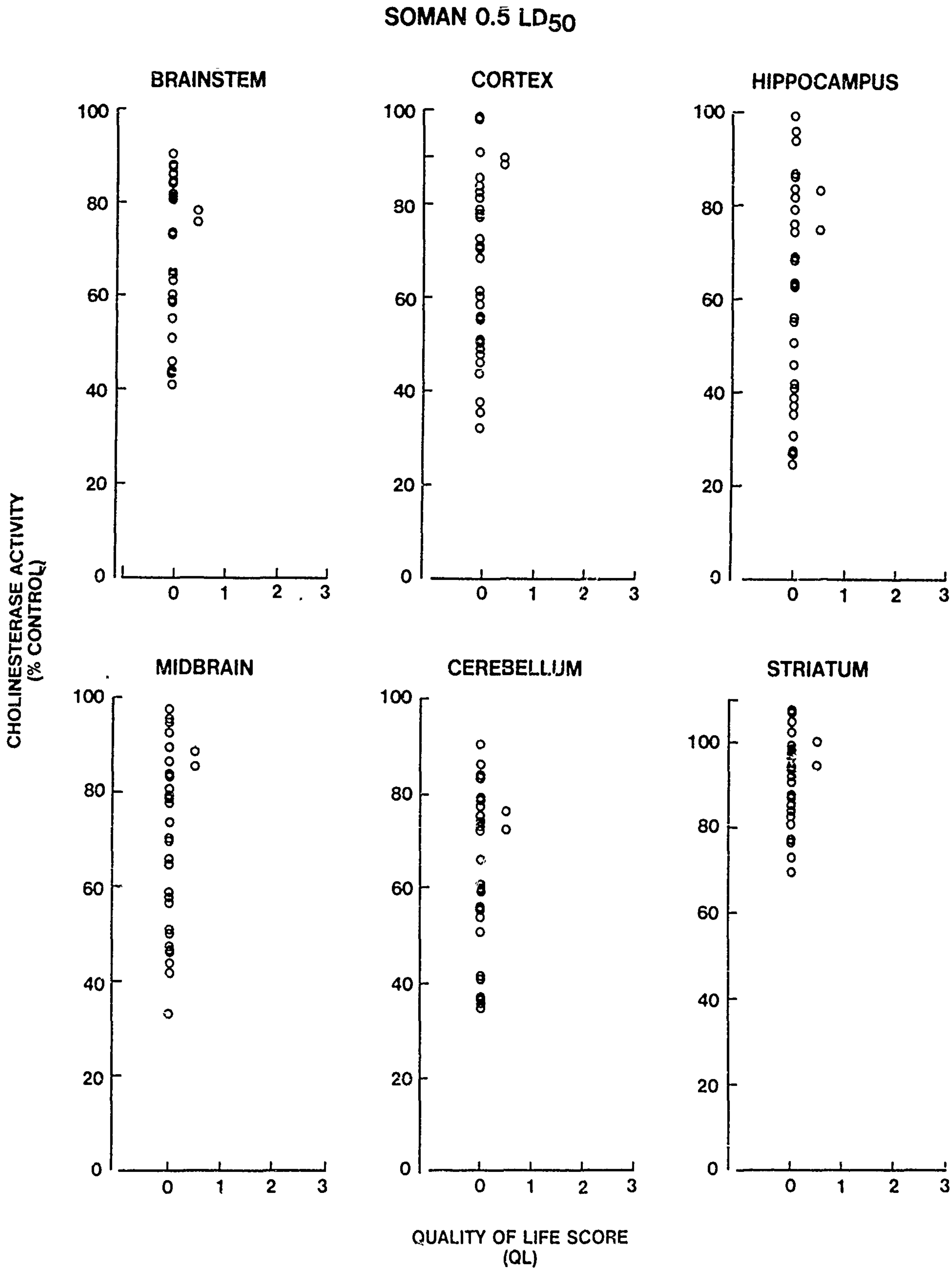
- Male, Sprague-Dawley rats, weighing 200-275 grams, were used.
- Rats were: (1) injected with a single subcutaneous dose of 0.3, 0.5, 0.6 or 0.7 LD_{50} of soman (LD_{50} =110 μg/kg).
- Rats were: (2) killed by decapitation at selected time points after soman administration.
- Rats were: (3) Scored for toxic signs just prior to termination according to the following "Quality of Life" (QL) categories, based on the behavioral code developed by Jovic (1974):
  - 0 = sign free
  - 1 = ataxia; muscle fasciculations; licking/chewing behavior
  - 2 = salivation; limb weakness with hindlimb splaying; tremors; jerking; convulsions
  - 3 = moribund with loss of righting reflex
- (occasionally intermediate scores, i.e., 0^+, 1^+, 2^+ were used).
- Brain regional ChE activity was determined in brainstem (BST), cortex (CTX), hippocampus (HIP), midbrain (MB), cerebellum (CB) and striatum (STR) by the automated colorimetric method of Groff et al (1976).

CORRELATION BETWEEN BRAIN REGIONAL ChE ACTIVITY AND QUALITY OF LIFE SCORE

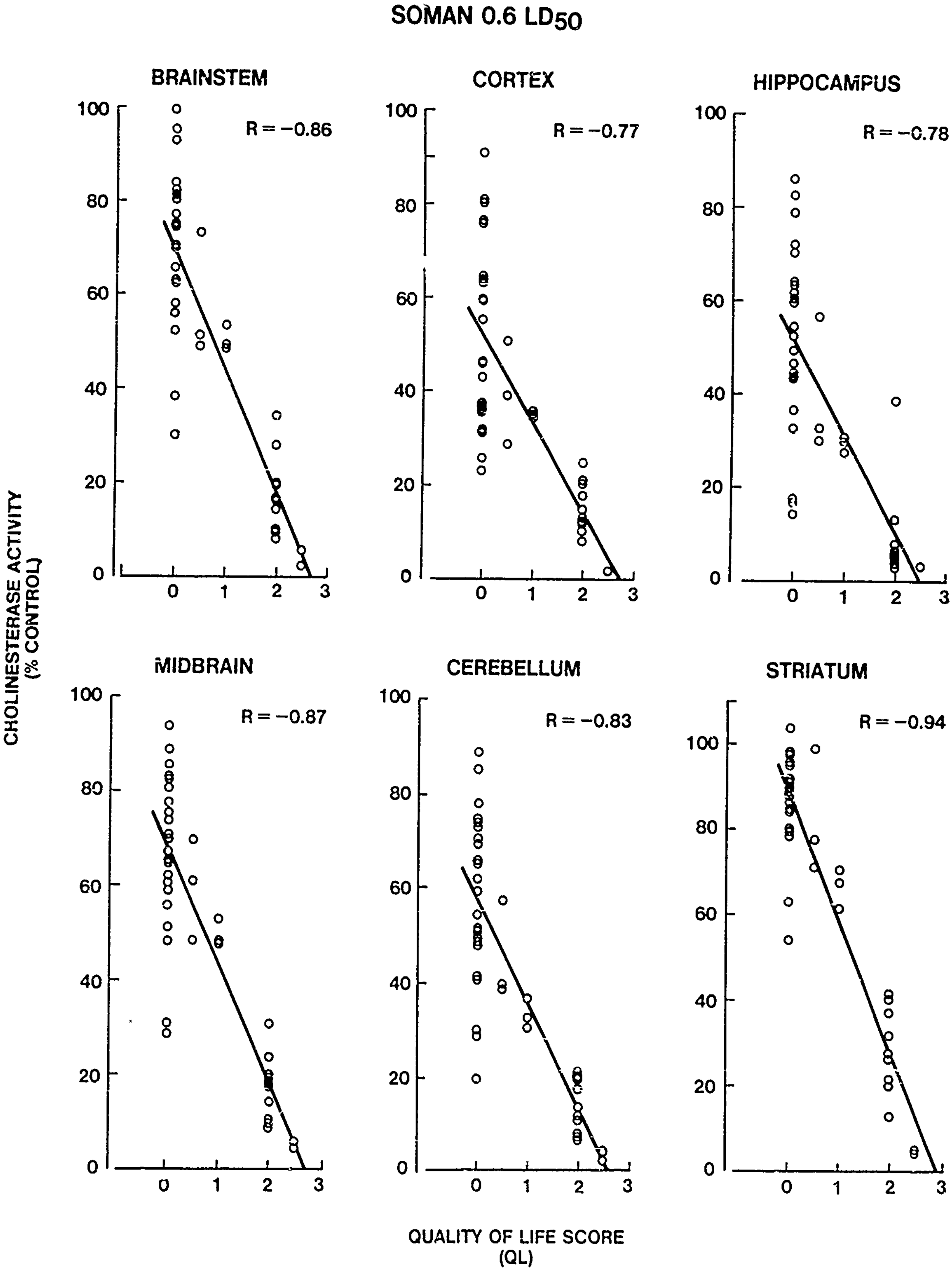


All animals (24/24) were sign-free (QL = 0)

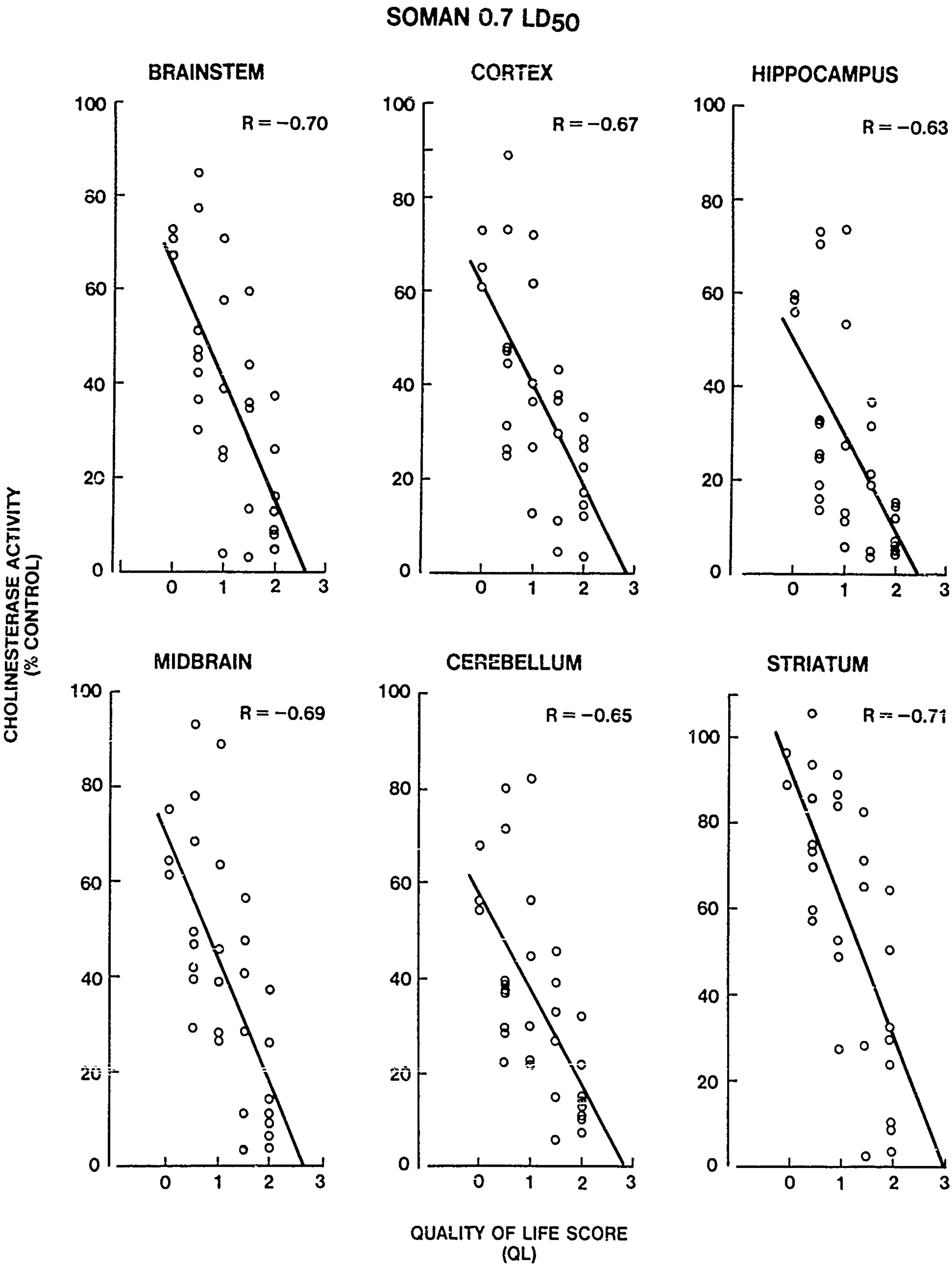
A-1151



Virtually all animals (28/30 were sign-free. Two animals showed minimal signs ($QL = 0^+$) but did not differ from sign-free animals in degree of inhibtion of brain regional ChE. Note the wider range of values.



Recognizable signs of toxicity were apparent in 52% (43/82) of the animals.



Recognizable signs of toxicity were apparent in 91% (29/32) of the animals.

Lines denote the least-squares linear regression fit for the data. R represents the coefficients of correlation for those regressions.

EFFECT OF VARIOUS DOSES OF SOMAN ON BRAIN REGIONAL ChE ACTIVITY (% CONTROL) WITH RESPECT TO QUALITY OF LIFE SCORE

DOSE (xLD_{50})	QL	N=	% CONTROL CHOLINESTERASE ACTIVITY BST	CTX	HIP	MB	CB	STR
0.3	0	24	91.2 ±1.7	96.4 ±2.2	89.2 ±1.7	88.1 ±1.0	81.2 ±1.7	102.8 ±4.7
0.5	0	30	70.0 ±3.0	65.1 ±3.4	59.4 ±4.3	68.5 ±3.4	61.9 ±3.8	91.4 ±1.9
	0+	2	76.9 —	89.2 —	78.6 —	88.6 —	74.0 —	97.5 —
0.6	0	25	68.6 ±3.6	52.2 ±3.9	52.0 ±4.1	67.1 ±3.3	58.5 ±3.6	85.7 ±2.1
	0+	3	57.7 ±7.8	39.4 ±6.3	39.6 ±8.5	59.8 ±6.2	45.7 ±6.1	82.5 ±8.4
	1	3	50.5 ±1.5	34.8 ±0.4	29.1 ±1.0	49.7 ±1.7	33.7 ±1.9	66.5 ±2.7
	2	11	17.7 ±2.4	15.0 ±1.6	6.6 ±0.9	17.3 ±2.0	13.9 ±1.6	27.3 ±2.8
	2+	2	4.0 —	1.8 —	3.2 —	5.0 —	3.8 —	4.8 —
0.7	0	3	70.6 ±1.7	66.6 ±3.6	57.9 ±1.1	67.2 ±4.2	59.3 ±4.2	93.6 ±2.4
	0+	9	51.6 ±6.1	48.4 ±7.1	34.1 ±7.4	52.9 ±7.4	42.5 ±6.6	76.6 ±5.2
	1	6	37.2 ±9.9	41.9 ±9.0	30.8 ±11.0	48.8 ±9.8	42.7 ±9.6	64.9 ±10.6
	1+	6	32.2 ±8.4	27.4 ±6.4	19.5 ±5.5	31.5 ±8.5	27.3 ±6.1	54.8 ±13.2
	2	8	16.2 ±3.8	20.0 ±3.4	8.6 ±1.6	15.4 ±3.9	15.3 ±2.8	27.6 ±7.5

CONCLUSIONS

- Single doses of soman at or below 0.5 LD_{50} are sign-free doses with a corresponding moderate inhibition of brain regional ChE activity.
- Single doses of soman greater than 0.5 LD_{50} produce a variety of recognizable signs of organophosphate intoxication and produce different degrees of marked inhibition of brain regional ChE activity.
- The magnitude of this marked inhibition correlates well with the severity of toxic signs, regardless of the sign-producing doses.
- A rough prediction of brain regional ChE activity may be possible by evaluating the severity of signs of soman toxicity.

REFERENCES

- Groff, W.A., Kaminskis, A. and Ellin, R.I.: Interconversions of cholinesterase enzyme activity units by the manual pH method and a recommended automated method. Clin. Toxicol. 9: 353-358 (1976).
- Jovic, R.C.: Correlation between signs of toxicity and some biochemical changes in rats poisoned with soman. Eur. J. Pharmacol., 25: 159-164 (1974).
- Shih, T.-M.: Effects of soman on blood and regional brain cholinesterase activities. Trans. Am. Soc. Neurochem., 14: 148 (1983).

EFFECTS OF SOMAN ON SCHEDULE-CONTROLLED BEHAVIOR

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ABSTRACT

Experiments were designed to examine the effects of soman on schedule-controlled performance. An ultimate goal of this research is to produce a "behavioral assay" with which to test organophosphorus agents and their antagonists.

Rats were trained to press a lever under a multiple fixed-ratio 25 fixed-interval 50-second (mult FR25 FI50-sec) schedule of food reinforcement. Soman, 70-90 ug/kg, s.c., suppressed to variable degrees the rate of responding in both components, with a slightly greater effect in the FI component. The pattern of responding under the FI schedule, however, was maintained until lever-pressing was almost completely suppressed. At the highest doses, soman occasionally caused tremors or mild tonic seizures with hindlimb abduction. The suppression of response rate was only loosely related to dose, but was highly correlated with inhibition of acetylcholinesterase (AChE) in all brain regions examined: cortex, striatum, hippocampus, hypothalamus and brainstem. Cortical AChE was inhibited to the highest degree and striatal AChE to the least. AChE in the gastrointestinal tract was not significantly inhibited, further suggesting that the behavioral deficit was centrally mediated.

In contrast, i.p. injection of either soman (10-40 ug/kg), neostigmine (75 ug/kg), or DFP (350 ug/kg) suppressed behavior and inhibited AChE in the gut, without affecting brain AChE. Peristaltic activity increased markedly, and likely caused gastrointestinal spasm. Injection of DFP, 500 ug/kg, s.c., inhibited AChE in both the brain and gut. The results indicate that inhibition of AChE in the gastrointestinal tract by certain anticholinesterase agents may be involved in the behavioral effects attributed to these drugs.

Attempts are underway to develop a within-subject behavioral assay, in which each animal can serve as its own control. This requires reproducible behavioral responses to soman. We showed previously that tolerance developed to the behavioral effects of i.p. soman if injections were made 3 or fewer weeks apart. Reproducible effects were seen when soman was injected i.p. at 5 week intervals. Since recovery of brain AChE activity following s.c. injection of soman also required about 3 weeks, we made repeated s.c. injections of behavior-suppressing doses of soman (70-90 ug/kg) at intervals of 5 weeks. Baseline performance, even following 6 injections, was not altered significantly, provided that convulsions did not occur. Reproducible degrees of suppression were obtained in a small percent of the animals, suggesting that within-subject testing of antagonists to the behavioral effects of soman is feasable, but may not be practical.

This work supported in part by the US Army Medical Research and Development Command under Contract DAMD17-82-C-2172.

INTRODUCTION

Soman is a potent and highly toxic organophosphate cholinesterase inhibitor. Sublethal doses inhibit open-field behavior and cause hypothermia, tremors, and hindlimb abduction. Our studies were initiated to examine the effects of soman on complex tasks involving schedule-controlled behavior.

METHODS

Adult male Sprague-Dawley rats, initially weighing 350-375g, were maintained at 80% of their free-feeding body weight and housed individually with water freely available.

Apparatus and procedures

Behavioral experiments were conducted in single-lever operant conditioning chambers and associated sound attenuating enclosures (Coulbourn Instruments). Programming was accomplished with a Digital System PDP-8 Computer and SKED software. Noyes food pellets (0.045 g) served as reinforcers.

The animals were trained to press a lever under a multiple fixed-ratio 25 fixed-interval 50-sec (FR25 FI50-sec) schedule of food reinforcement. (Under the FR25 schedule, the rat must press a lever 25 times to receive a food pellet. Under the FI50-sec schedule, a food pellet only becomes available 50 seconds after the previous pellet was received. The rat then needs only to press the lever once to obtain the reward.)

Six 10-min FR25 and FI50-sec components alternated successively throughout the session, and the component in which the session was initiated was determined randomly.

For each session, we monitored the number of bar-presses per second under the FI and FR components. In addition, the computer generated a "quarter-life" value, which represents the percent of the 50 second interval elapsing before 25% of the responses are made. A high quarter life value indicates low responses during the early portion of the interval and higher rates during the later portion; a pattern normally associated with an FI schedule.

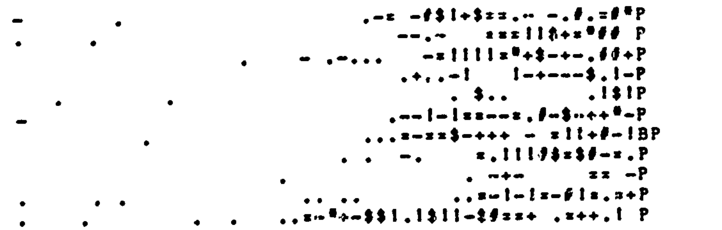
Acetylcholinesterase assay

AChE was assayed by the spectrophotometric method of Ellman et al. Brain and gut were homogenized in 100 mM phosphate buffer (pH 8.0) at a concentration of 100 mg/ml. An 8 ul volume of 75 mM acetylthiocholine was added to a microcuvette containing 1.02 ml phosphate buffer, 8 ul brain homogenate and 40 ul of 10 mM dithiobisnitrobenzoic acid. The change in absorbance was measured at 412 u.

Drugs

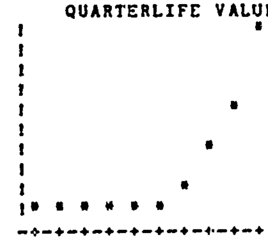
All drugs were dissolved in saline immediately prior to use such that the volume of each injection was 1.0 ml/kg body weight. Drugs were injected subcutaneously unless otherwise noted.

```
FFFFFF  IIIII
F         I
FFFF      I
F         I
F       IIIII
```



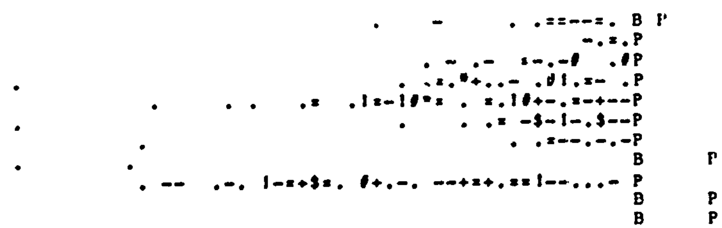
MEAN TIME FROM END OF FI TO PRESS 0.40
TOTAL NUMBER OF FI'S THAT OCCURRED 11
FI RATE PER SECOND IS 1.22

QUARTERLIFE VALUES FOR FI



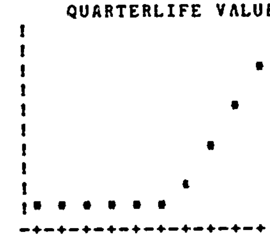
QUARTER LIFE VALUE IS 70%

```
FFFFFF  IIIII
F         I
FFFF      I
F         I
F       IIIII
```



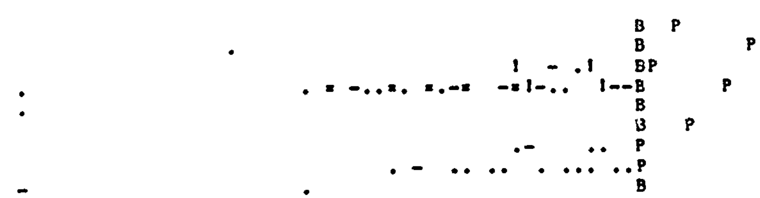
MEAN TIME FROM END OF FI TO PRESS 2.10
TOTAL NUMBER OF FI'S THAT OCCURRED 11
FI RATE PER SECOND IS 0.50

QUARTERLIFE VALUES FOR FI



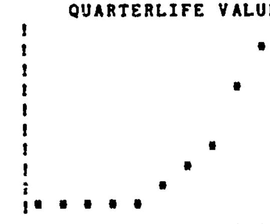
QUARTER LIFE VALUE IS 64%

```
FFFFFF  IIIII
F         I
FFFF      I
F         I
F       IIIII
```



MEAN TIME FROM END OF FI TO PRESS 14.12
TOTAL NUMBER OF FI'S THAT OCCURRED 9
FI RATE PER SECOND IS 0.17

QUARTERLIFE VALUES FOR FI



QUARTER LIFE VALUE IS 64%

```
FFFFFF RRRRR
F      R    R
FFFF   RRRRR
F      R  R
F      R    R

MEAN TIME FOR FR TO BE COMPLETED        7.60
TOTAL NUMBER OF FR IN THIS COMPONENT     76
FR RATE PER SECOND IS        3.16
```

```
FFFFFF RRRRR
F      R    R
FFFF   RRRRR
F      R  R
F      R    R

MEAN TIME FOR FR TO BE COMPLETED        9.15
TOTAL NUMBER OF FR IN THIS COMPONENT     64
FR RATE PER SECOND IS        2.66
```

```
FFFFFF RRRRR
F      R    R
FFFF   RRRRR
F      R  R
F      R    R

MEAN TIME FOR FR TO BE COMPLETED        70.44
TOTAL NUMBER OF FR IN THIS COMPONENT      5
FR RATE PER SECOND IS        0.20
```

COMPUTER PRINTOUT OF PERFORMANCE UNDER A
MULTIPLE FIXED-INTERVAL 50-SEC / FIXED-RATIO 25 SCHEDULE
OF FOOD REINFORCEMENT FOLLOWING INJECTION OF SOMAN (75 ug/kg, S.C.)

The alternate FI50-sec and FR25 components are grouped in the upper and lower figures, respectively. Soman, 75 ug/kg, s.c., was injected immediately prior to starting the session. Drug effects did not begin for about 20 min, so that the first cycle of each component indicates the control pattern of responding.

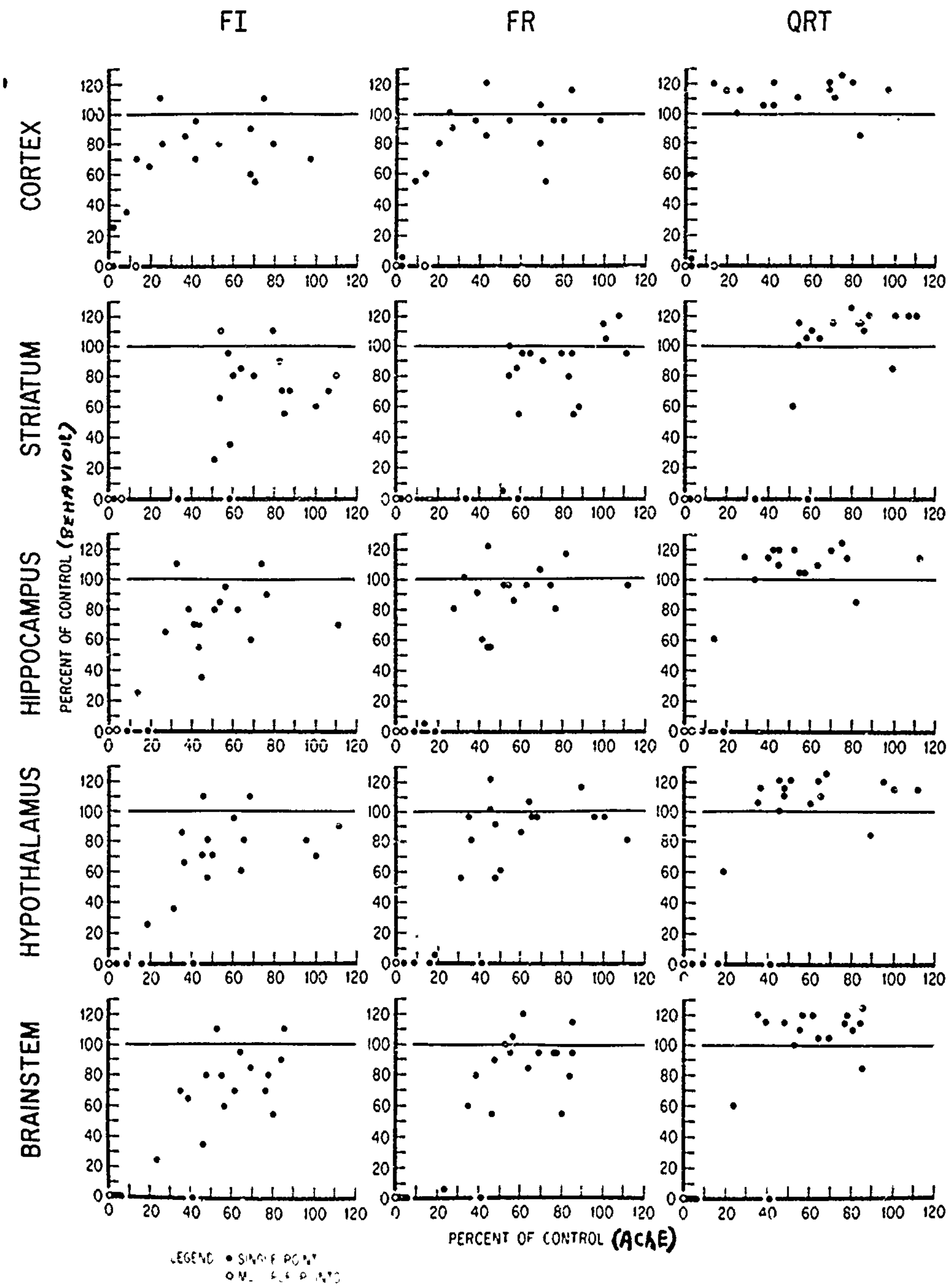
Note in the upper figure the progressive decrease in response rate along with the delay in obtaining the pellet at the end of the 50-second interval. In contrast, quarter life value was not significantly decreased, indicating that the pattern of responding was maintained.

Response rate also decreased progressively during the FR25 component (lower figure).

EFFECT OF SOMAN ON PERFORMANCE

Dose	N	FI presses/ second	FI QRT-Life[a]	FR presses/ second
		Baseline Values[b]		
		0.74 ± 0.6	48 ± 1	1.1 ± 0.6
		Percent of Baseline		
70	6	96 ± 9	110 ± 5	100 ± 6
75	3	59 ± 13	114 ± 5	65 ± 14
80	9	46 ± 13	54 ± 16	34 ± 14
90	7	57 ± 14	82 ± 14	59 ± 17

a. Quarter-life value (% of FI interval which elapsed before 25% of the bar-presses were completed).
b. Average of three days prior to drug for each of 25 rats.


FI
FR
QRT
CORTEX
STRIATUM
HIPPOCAMPUS
HYPOTHALAMUS
BRAINSTEM
PERCENT OF CONTROL (BEHAVIOR)
PERCENT OF CONTROL (AChE)
0 20 40 60 80 100 120

EFFECT OF SOMAN ON BRAIN ACHE ACTIVITY

Dose	N	Cortex	Striatum	Hippocampus	Hypothalamus	Brainstem
				Baseline Values[a]		
		4.5 ± 0.4	38.3 ± 3.0	8.7 ± 0.4	8.8 ± 0.5	10.5 ± 0.7
				Percent of Baseline		
70	6	67 ± 11	89 ± 9	66 ± 12	74 ± 10	73 ± 6
75	3	44 ± 12	68 ± 9	51 ± 6	48 ± 10	61 ± 10
80	9	25 ± 10	43 ± 12	29 ± 10	31 ± 11	36 ± 11
90	7	20 ± 9	50 ± 15	27 ± 10	34 ± 9	27 ± 9

a. umoles substrate hydrolyzed/min/g tissue.

The behavioral effects of soman were apparent at doses between 70 and 90 ug/kg. Above the higher dose many animals either died or developed convulsions. Within the 70-90 ug/kg range, however, there was considerable variability in response. This is reflected in the variability of brain AChE inhibition at these doses. When the level of behavior suppression is compared with the degree of AChE inhibition, however, a high degree of correlation is obtained.

EFFECT OF DRUGS ON RESPONDING AND AChE ACTIVITY

Drug	Dose ug/kg	N	Responses/second FR 25	FI 50	AChE Activity Cortex	Hippocamp	Hypothal	Ileum	Plasma
——		10	2.2 ± 0.2	1.1 ± 0.2	4.5 ± 0.6	8.6 ± 0.4	8.5 ± 0.5	2.9 ± 0.6	4.8 ± 1.0
			Percent of Baseline						
Soman	10, ip	4	50 ± 6	45 ± 6	——	——	——	31 ± 12	71 ± 9
Soman	40, ip	6	0 ± 0	0 ± 0	110 ± 12	88 ± 8	94 ± 9	17 ± 2	42 ± 6
Soman	80, sc	9	34 ± 14	46 ± 13	25 ± 10	29 ± 10	31 ± 11	92 ± 6	4 ± 1
Neost	75, ip	4	0 ± 0	0 ± 0	98 ± 7	86 ± 10	91 ± 13	55 ± 10	38 ± 5
DFP	500, sc	3	0 ± 0	0 ± 0	0 ± 0	2 ± 1	5 ± 2	0 ± 0	2 ± 2
DFP	350, ip	3	24 ± 7	12 ± 2	85 ± 6	72 ± 5	——	3 ± 3	4 ± 3

AChE activity reported as umoles substrate hydrolyzed/g tissue/min.
Neost = Neostigmine

EFFECTS OF CHOLINESTERASE INHIBITORS INJECTED BY DIFFERENT ROUTES ON SCHEDULE-CONTROLLED BEHAVIOR AND ON ACHE ACTIVITY IN BRAIN AND GUT

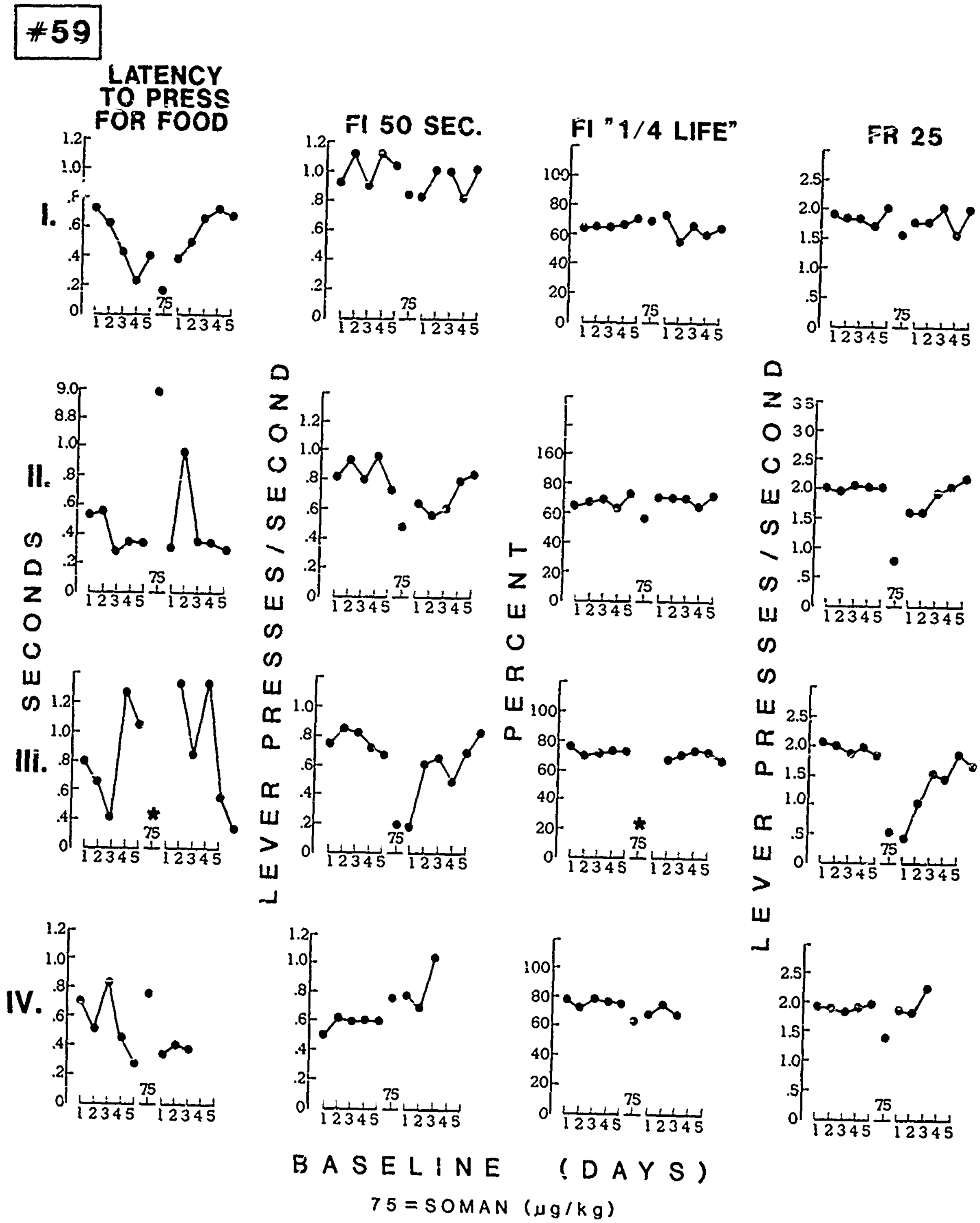
Intraperitoneal injection of soman, 10 and 40 ug/kg, produced a dose-related suppression of responding. The only overt symptom was diarrhea at the higher dose. At these doses, injected i.p., soman did not affect brain AChE but markedly reduced AChE in the gut and caused increased peristaltic activity. In contrast, s.c. doses of soman which affected behavior caused a significant reduction of AChE activity in brain, but not in gut.

Neostigmine, which does not readily enter the brain, also suppressed behavior when injected i.p. Again, AChE activity was reduced in the gut, but not in the brain. As with soman i.p., neostigmine caused diarrhea.

DFP, administered i.p., suppressed behavior and inhibited AChE in gut to a greater extent than in brain. When injected s.c., AChE was inhibited in both brain and gut.

These experiments demonstrate that inhibition of AChE activity in the gut can suppress schedule-controlled behavior maintained by food reinforcement. This can occur in the absence of any significant reduction in brain AChE activity and may be related to gastrointestinal spasm.

Subcutaneous injection of soman, possibly because of its rapid penetration into the brain, inhibits brain AChE at doses which only marginally affect the gut. In view of the relatively selective central effects of soman, it may be a better agent than DFP for behavioral studies of central cholinergic function.



Data for Rat 59 are shown in more detail in this Figure. Disruption of FI performance following soman is indicated by an increase in the latency to obtain a food pellet when it becomes available, overall slowing of FI rate, and disruption of quarter life. Soman led to slowing of the FR rate, due partly to an increase in post-pellet pausing and to disruption of the ratio run (see cumulative records). Responses in both components returned to baseline levels within 1-3 sessions. For Rat 59, FR 25 performance was more consistently affected by repeated injection of soman than was FI performance.

PERCENT OF BASELINE RESPONSE RATES
FOLLOWING REPEATED INJECTIONS OF SOMAN AT
5-WEEK INTERVALS IN 9 OF 12 RATS SURVIVING THE FIRST INJECTION

	REPLICATION									
	1		2		3		4		5	
Rat	FI	FR	FI	FR	FI	FR	FI	FR	FI	FR
58	83	102	87	103	101	98	107	92	0	0
Dose[a]	75		80		85[b]		40[b]		40[b]	
59	83	81	69	38	31	32	127	75		
Dose	75		75		75		75			
60	120	99	65	93	44	62	27	38		
Dose	75		80		85		85			
104	30	14	94	66	0	0[c]				
Dose	75		75		75					
105	120	106	91	104	97	78	118	94		
Dose	75		80		85		85			
106	0	0	107	97	94	91	37	0		
Dose	75		72		78		80			
107	0	0	89	117	33	0	63	52		
Dose	80		80		85		85			
115	122	154	114	82	71	81	0	0	0	0[c]
Dose	80		80		85		85		85	
125	108	89	76	108	86	89	29	59	121	102
Dose	75		85		90		90		90	

a. Dose of soman, ug/kg, s.c.

b. Soman administered on three successive days.

c. Lethal seizure.

Table 1 summarizes the performance of nine animals who survived the first injection of soman. Two additional animals died following repeat administration of soman. Interestingly, their death was heralded by marked deterioration of FI performance over time. Baseline performance of the other animals remained intact. At this time, 7 of 12 animals still are being followed, with four beginning to reveal a pattern of replicable suppression of response rates following soman administration, although much variability in the data is present. One animal who failed to reveal response suppression to soman revealed suppression when lesser doses of soman were administered on consecutive days.

CONCLUSION

The present study, as well as data collected for numerous other animals, shows that it is possible to repeatedly administer low but effective doses of soman without disrupting baseline schedule performance. It is apparent, however, that a relatively large number of animals is needed to obtain the number of animals necessary for behavioral analyses of soman. In the present study, 5 of 12 animals did not survive. Of the survivors, four are just beginning to reveal consistent responses to soman, although the degree of variability present is greater than that normally encountered in the experimental analysis of other compounds. Further parametric manipulations are planned to increase the "yield" of experimental animals and we are exploring the idea of introducing somewhat lower doses of soman over successive days to increase the percentage of animals which reveal replicable responses to soman and decrease the percent which suffer lethal siezure.

NEUROCHEMICAL CORRELATES OF ACUTE SOMAN INTOXICATION: NEURONAL RNA CHANGES IN CEREBROCORTICAL, THALAMIC & MESENCEPHALIC BRAIN COMPARTMENTS

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QUANTITATIVE AZURE B CYTOPHOTOMETRIC ANALYSES OF RIBONUCLEIC ACID (RNA) RESPONSES OF INDIVIDUAL NEURONS IN SPECIFIC CEREBRO-CORTICAL, THALAMIC AND MESENCEPHALIC-RETICULAR BRAIN REGIONS WERE MONITORED IN RATS FOLLOWING ACUTE SINGLE SUBCUTANEOUS INJECTIONS IN SOMAN (PINACOLYL METHYLPHOSPHONOFLUORIDATE) USING 0.5, 0.9 OR 1.5 LD_{50} DOSAGES. ANIMAL SACRIFICE WAS BY DECAPITATION WITHOUT ANESTHESIA 30 MIN POST-INJECTION EXCEPT FOR THE 1.5 LD_{50} GROUP WHICH WAS KILLED UPON IMPENDING DEATH (RESPIRATORY FAILURE) OR AT 10 MIN. CORRELATIVE DATA WERE ALSO OBTAINED ON SOMAN-INDUCED CHANGES IN BRAIN AND ERYTHROCYTE ACETYLCHOLINESTERASE (ACHE) ACTIVITY.

A NON-DOSE DEPENDENT SUPPRESSION OF RNA WAS EVIDENCE IN CEREBROCORTICAL AND STRIATAL NEURONS FOLLOWING ACUTE SOMAN TOXICATION INDICATING NON-CHOLINERGIC MECHANISMS APPEAR TO BE INVOLVED IN MEDIATING NUCLEIC ACID CHANGES IN THESE FOREBRAIN REGIONS. IN CONTRAST, SEVERAL SUBCORTICAL CHOLINOCEPTIVE BRAIN REGIONS EXHIBITED A COMPLEX BUT DOSE-RELATED RNA RESPONSE PATTERN. FOR EXAMPLE, IN SPECIFIC BRAIN COMPARTMENTS CONTAINING NEURONS WHICH PREDOMINANTLY EXHIBIT EXCITATORY RESPONSES TO IONTOPHORETIC ACH (E.G., VENTRAL BASAL NUCLEAR COMPLEX OF THE THALAMUS AND THE VENTROTEGMENTAL AND CUNEIFORM NUCLEI OF THE RETICULAR FORMATION) SUBLETHAL DOSES OF SOMAN DO IN FACT ELICIT NEURONAL RNA AUGMENTATION AS ONE WOULD ANTICIPATE; NEAR-LETHAL OR LETHAL DOSAGES, ON THE OTHER HAND, ELICIT A DOSE-DEPENDENT SUPPRESSION OF RNA IN THESE BRAIN REGIONS. INTERESTINGLY, OTHER BRAIN SITES CONTAINING ACH-INHIBITORY NEURONS SUCH AS THE THALAMIC NUCLEUS RETICULARIS EXHIBIT AN OPPOSITE RNA RESPONSE PATTERN TO THAT OBSERVED IN ACH-EXCITATORY BRAIN REGIONS, I.E., AN RNA SUPPRESSION WITH A DOSAGE OF 0.5 LD_{50} AND A MARKED RNA AUGMENTATION WITH 1.5 LD_{50}.

THE OVERALL DATA INDICATE THAT METABOLIC CORRELATES OF ENHANCED ACTIVATION OF CHOLINERGIC BRAIN STEM NUCLEI ARE EVIDENCED ONLY WITH SUBLETHAL DOSAGES OF SOMAN WHEREAS LETHAL DOSAGES EFFECT A DISRUPTION OR IMPAIRMENT OF EXCITATORY-INHIBITORY NEUROTRANSMITTER FUNCTIONING IN THALAMIC NUCLEI AND THE MESENCEPHALIC RETICULAR FORMATION. (SUPPORTED BY USAMRDC GRANT DAMD 17-81-C-1202).

METHODS

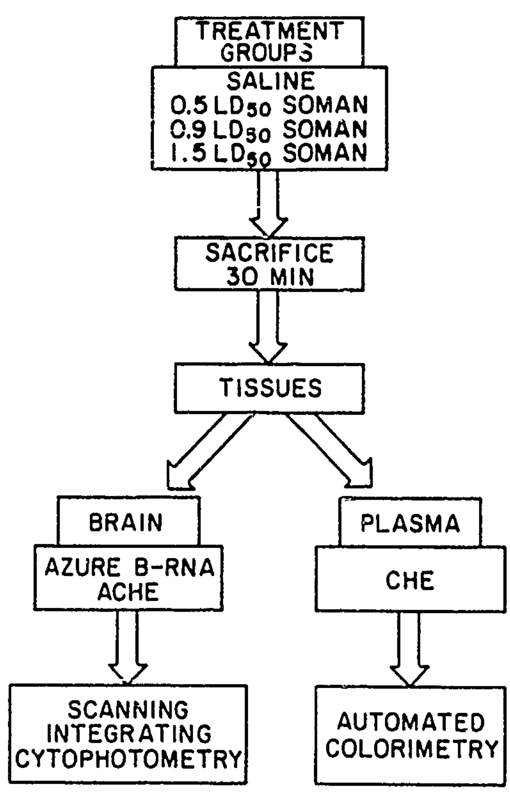
- ANIMAL MODEL: MALE SPRAGUE-DAWLEY RAT
- SOMAN DOSAGES: 0.5, 0.9 AND 1.5 LD_{50} SC
- BRAIN REGIONS ANALYZED:

  CEREBRAL CORTEX
  STRIATUM
  THALAMUS
  RETICULAR FORMATION
  SUBSTANTIA NIGRA

- MAJOR PARAMETERS ASSAYED/ANALYTICAL METHOD:

  NEURONAL RNA/SCANNING-INTEGRATING CYTOPHOTOMETRY
  BRAIN ACHE/SCANNING-INTEGRATING CYTOPHOTOMETRY
  PLASMA CHE/AUTOMATED COLORIMETRY

METHODS



AIMS

1) TO CHARACTERIZE THE NATURE OF SOMAN-INDUCED IMPAIRMENT IN REGULATORY ASPECTS OF NUCLEIC ACID METABOLISM IN DISCRETE SUBCORTICAL BRAIN COMPARTMENTS WHICH PREDOMINANTLY EXHIBIT EITHER EXCITATORY OR INHIBITORY RESPONSES TO ACH.

2) TO IDENTIFY SPECIFIC CHOLINERGIC COMPARTMENTS IN THALAMIC, MESENCEPHALIC AND OTHER SUBCORTICAL BRAIN REGIONS WHICH CONSTITUTE PRIMARY TARGET SITES OF SOMAN-INDUCED NEUROTOXICITY.

RESULTS

1) SOMAN AT ALL DOSAGES MARKEDLY INHIBITED PLASMA CHE, WHEREAS A DOSE-DEPENDENT DEPRESSION IN BRAIN ACHE WAS EVIDENCED.

2) MARKED REGIONAL DIFFERENCES IN BOTH THE PATTERN AND SEVERITY OF NEURONAL RNA RESPONSES WERE EVIDENCED IN CORTICAL AND SUBCORTICAL BRAIN REGIONS (TABLE 1, FIGURES 3-7).

3) WITH SUBLETHAL DOSAGES OF SOMAN (0.5 LD_{50}) BRAIN SITES CONTAINING PREDOMINANTLY ACH EXCITED NEURONS EXHIBITED SIGNS OF EXCITATION (INCREASED RNA LEVELS), I.E., THE THALAMIC VENTROBASAL NUCLEAR COMPLEX AND THE MESENCEPHALIC NUCLEUS CUNEIFORMIS.

4) IN THE NUCLEUS RETICULARIS, WHERE ACH ACTS AS AN INHIBITORY TRANSMITTER, A SUBLETHAL SOMAN CHALLENGE ELICITED AN RNA DEPLETION.

5) NEAR-LETHAL AND LETHAL SOMAN DOSAGES EFFECTED AN OPPOSITE PATTERN OF RNA RESPONSES IN VBC, NC AND NR COMPARTMENTS TO THAT OBSERVED WITH ASYMPTOMATIC DOSAGES.

6) NEURONS COMPRISING THE PARS COMPACTA OF THE SUBSTANTIA NIGRA, A MAJOR SITE OF DOPAMINE SYNTHESIS, EXHIBITED A DOSE-DEPENDENT RNA DEPLETION.

7) IN SEVERAL BRAIN REGIONS (PYRIFORM CORTEX, LATERAL THALAMIC NUCLEUS AND THE RETICULAR VENTROTEGMENTAL NUCLEUS), NEURONAL RNA LEVELS WERE UNAFFECTED WITH A 0.5 LD_{50} DOSE AND DEPRESSED FOLLOWING 0.9 AND 1.5 LD_{50} DOSAGES.

A-1172

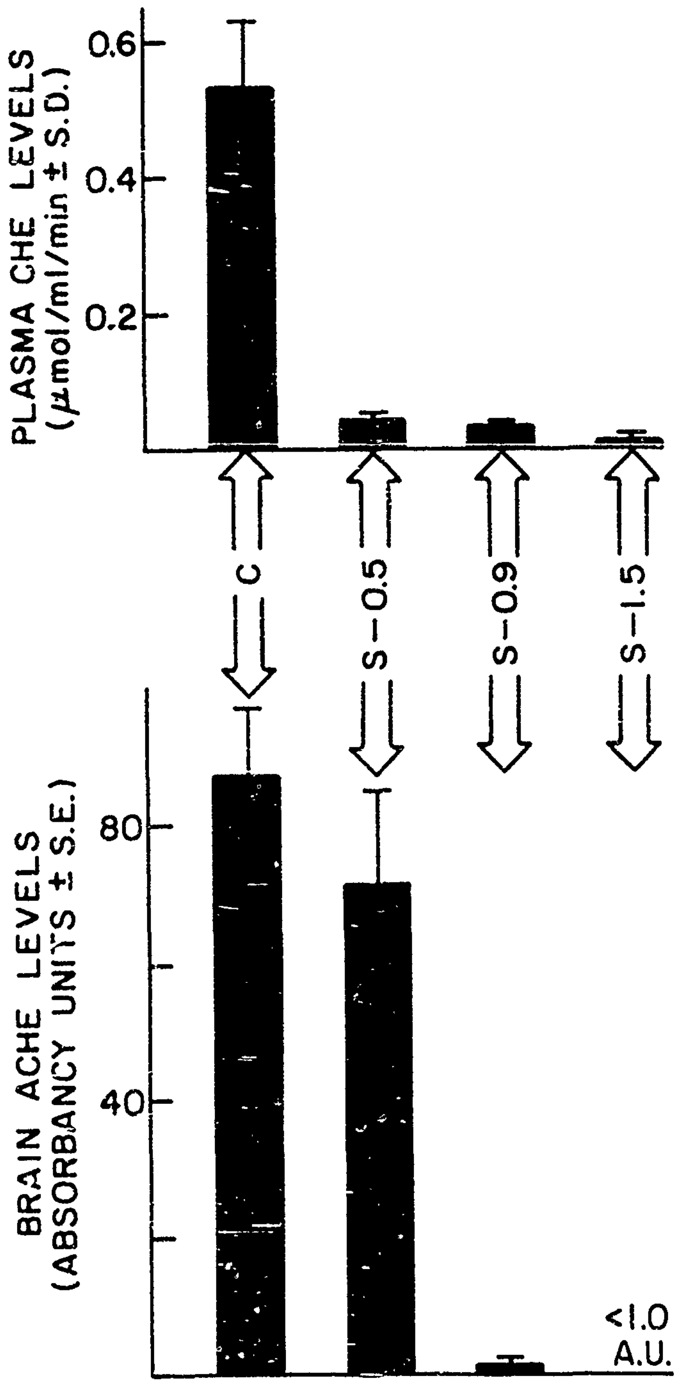
TABLE 1

DOSE-DEPENDENT CHANGES IN NEURONAL RNA CONTENT IN VARIOUS CNS COMPARTMENTS OF SOMAN INTOXICATED RATS

Brain Region	Neuronal RNA Content (A.U.)[1] Control	$0.5 LD_{50}$	$0.9 LD_{50}$	$1.5 LD_{50}$
Cerebral Cortex				
Sensorimotor	153 ± 4^{A}	119 ± 3^{C}	119 ± 2^{C}	129 ± 3^{B}
Pyriform	104 ± 2^{A}	$102 \pm 2^{A,B}$	98 ± 2^{B}	90 ± 2^{C}
Striatum	103 ± 2^{A}	95 ± 2^{B}	88 ± 2^{C}	87 ± 1^{C}
Thalamus				
Ventrobasal nuclear complex	77 ± 2^{B}	82 ± 1^{A}	65 ± 1^{C}	59 ± 1^{D}
Nucleus reticularis	85 ± 1^{B}	81 ± 2^{C}	$82 \pm 1^{B,C}$	95 ± 2^{A}
Lateral nucleus	91 ± 2^{A}	91 ± 2^{A}	83 ± 2^{B}	84 ± 2^{B}
Midbrain Reticular Formation				
Nucleus cuneiformis	116 ± 3^{B}	133 ± 3^{A}	99 ± 2^{C}	73 ± 2^{D}
Ventrotegmental nucleus	114 ± 3^{A}	112 ± 3^{A}	88 ± 3^{B}	74 ± 2^{C}
Substantia Nigra				
Pars compacta	136 ± 3^{A}	129 ± 2^{B}	120 ± 2^{C}	101 ± 2^{D}
Pars reticulata	82 ± 2^{B}	82 ± 2^{B}	94 ± 2^{A}	85 ± 2^{B}

[1] RNA content in Absorbancy Units ± Standard Error. Each value represents the average of 100 individual cytophotometric meausrements. For each brain region, means with different superscripts are significant, $P < 0.05$. Means ranked highest to lowest, the highest value desiganted as (A).

DOSE DEPENDENT CHANGES IN PLASMA CHOLINESTERASE AND BRAIN ACETYLCHOLINESTERASE LEVELS WITH ACUTE SOMAN TOXICATION



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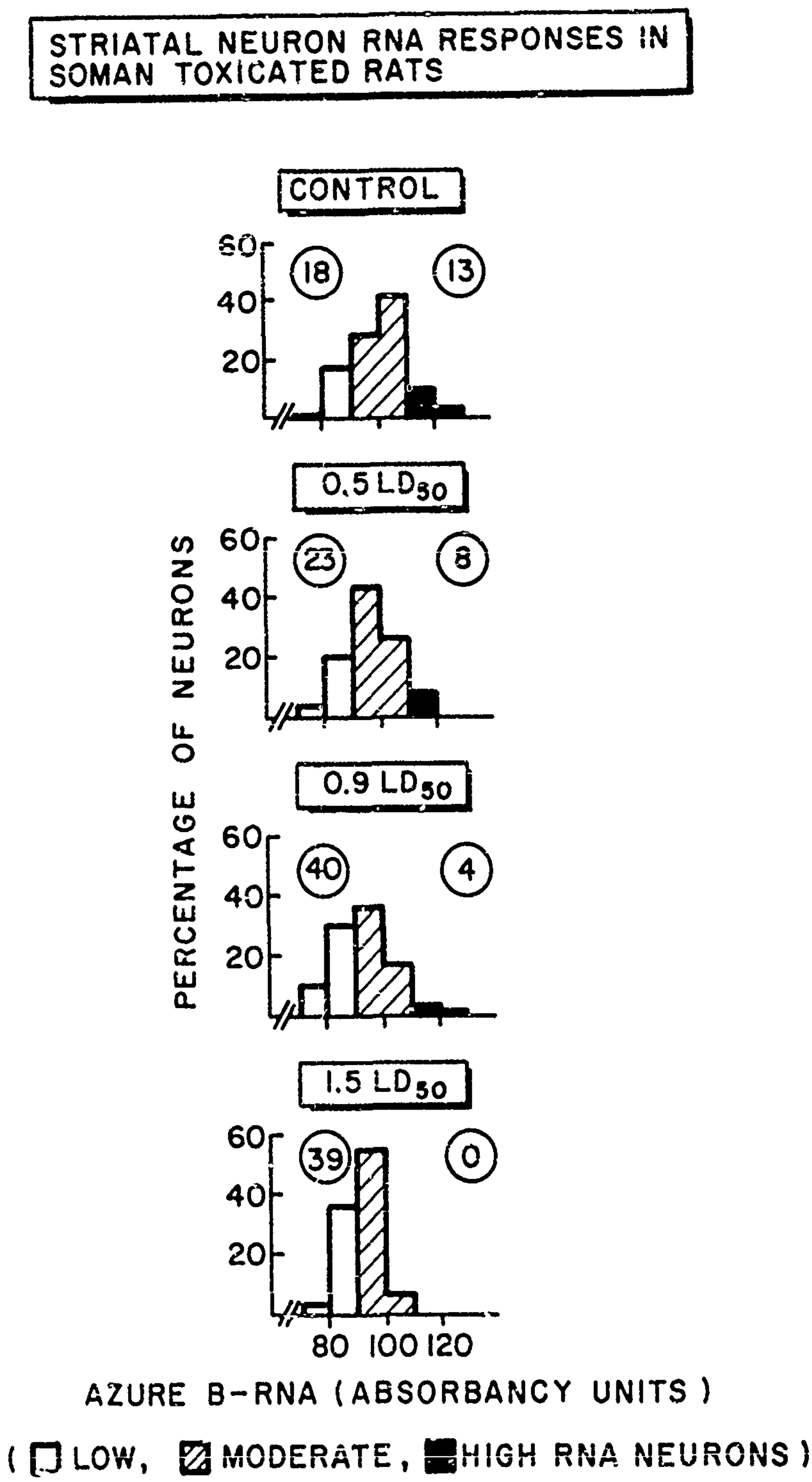
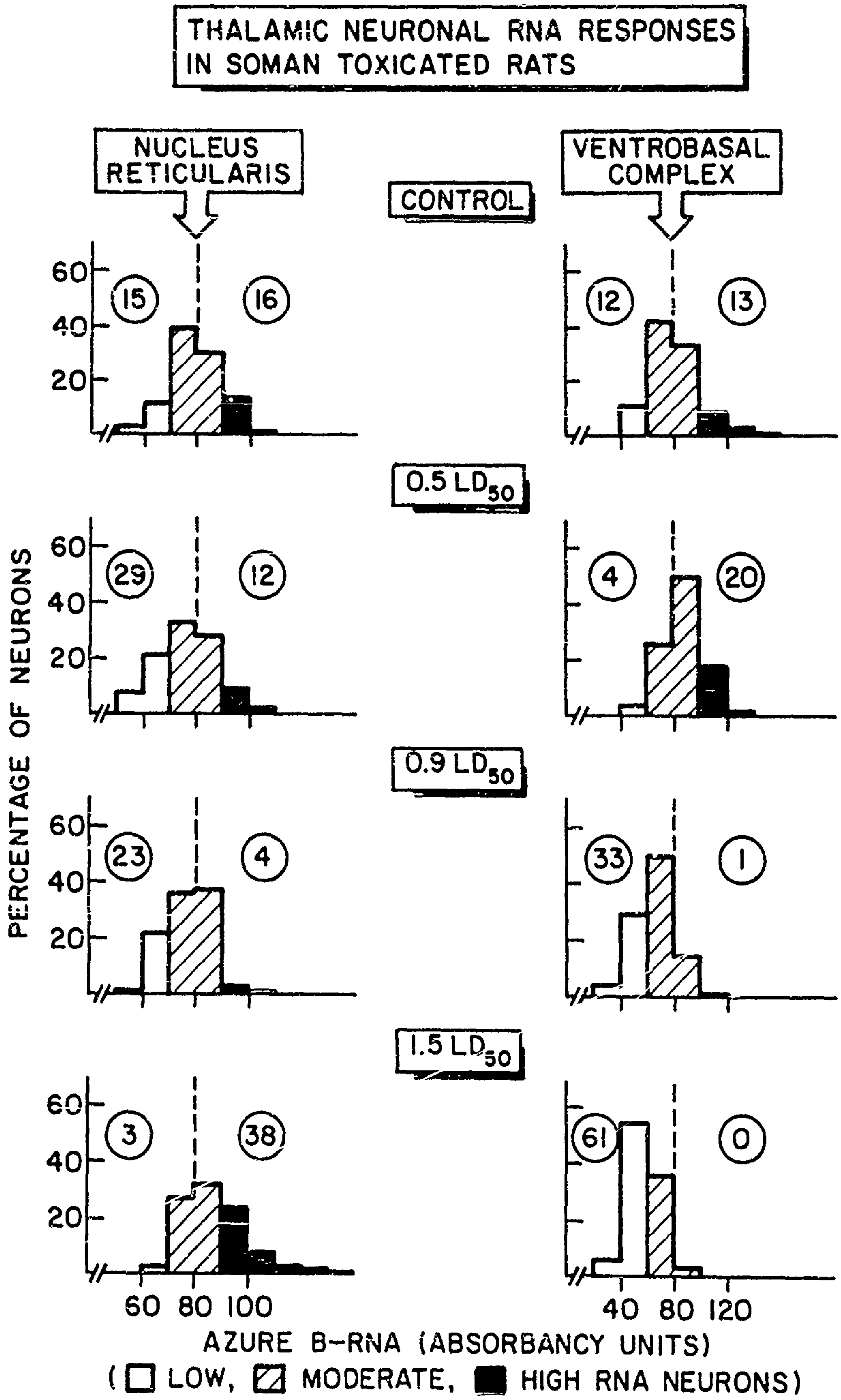
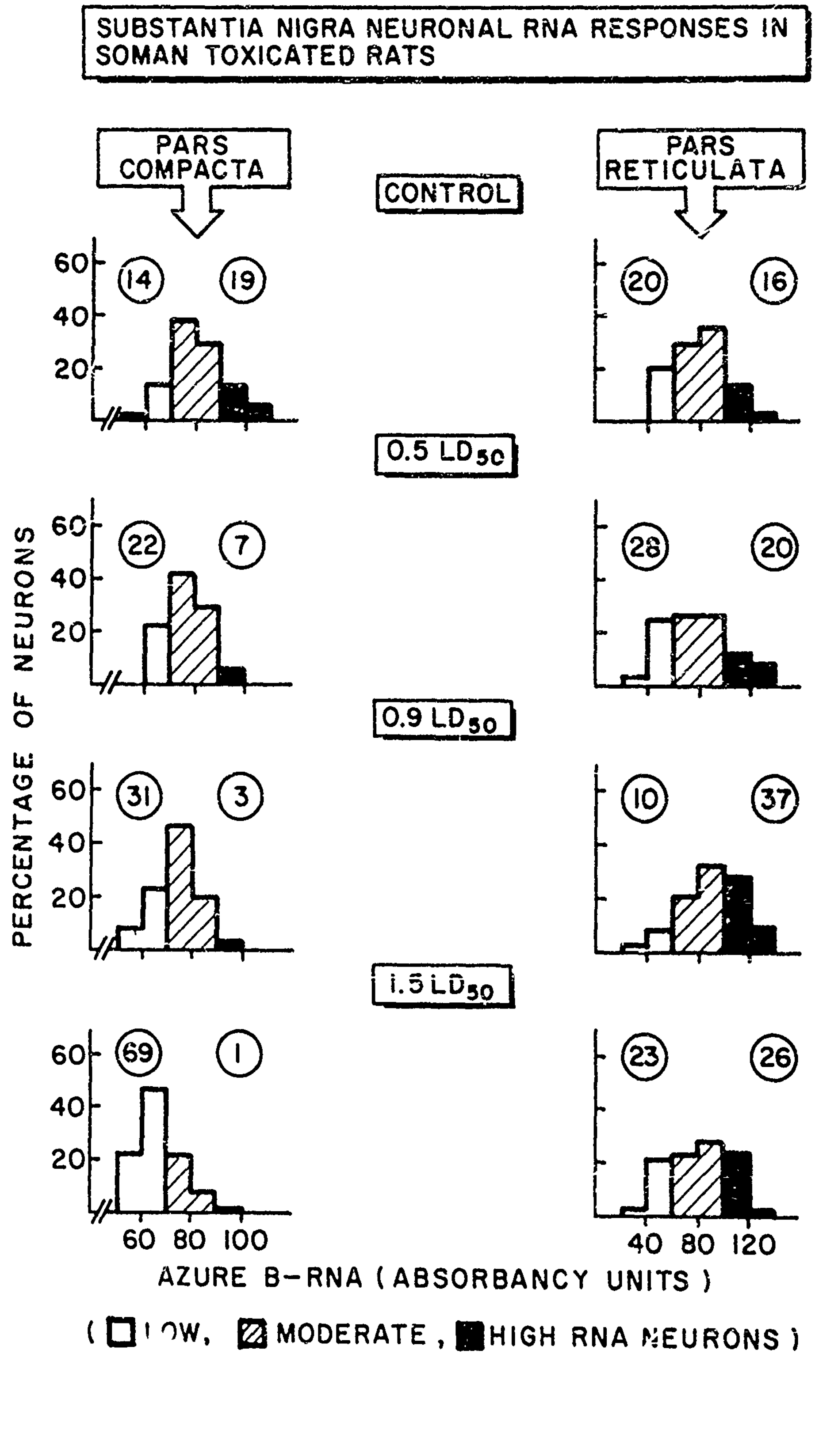
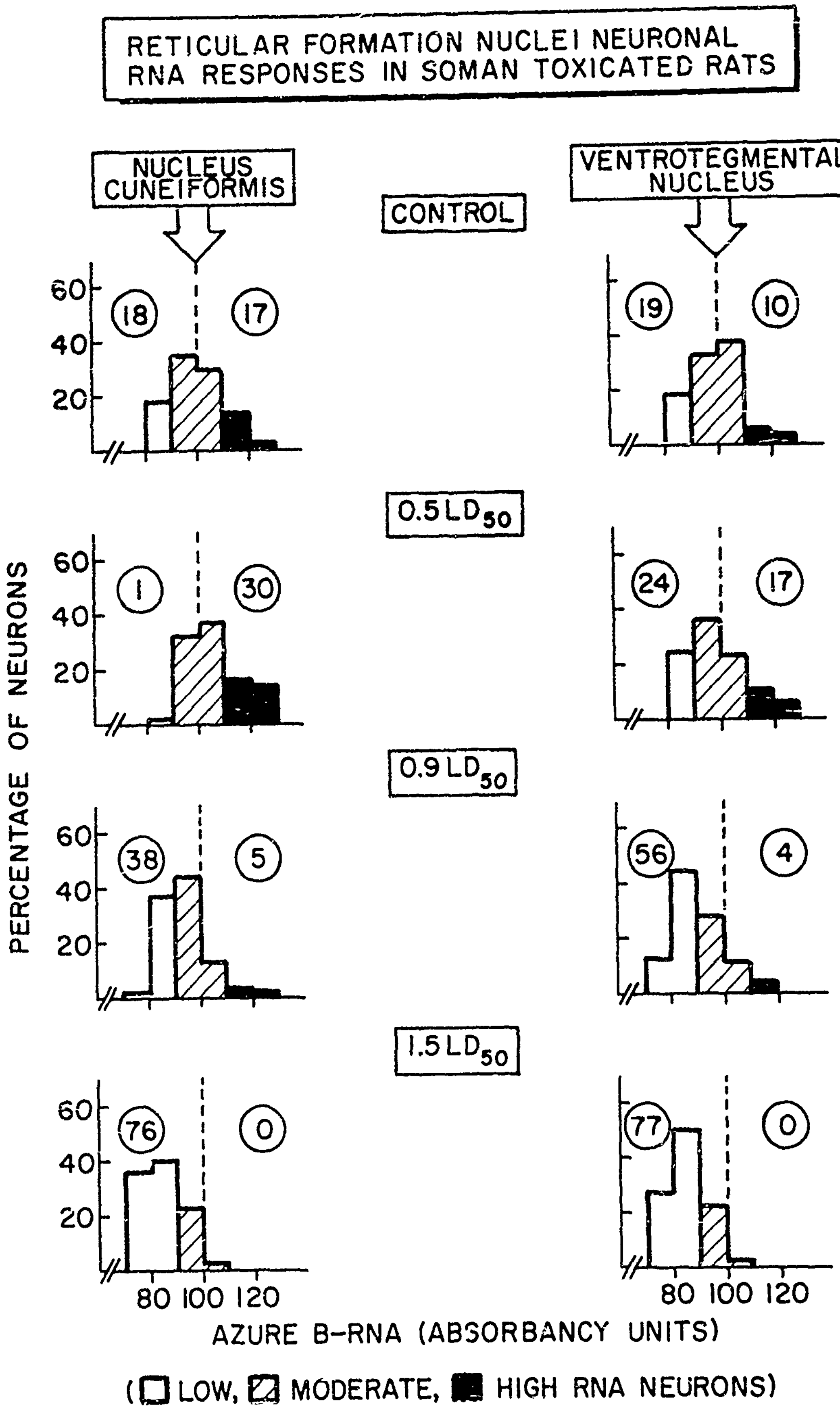

STRIATAL NEURON RNA RESPONSES IN SOMAN TOXICATED RATS
CONTROL
18
13
0.5 LD50
23
8
0.9 LD50
40
4
1.5 LD50
39
0
60
40
20
80 100 120
PERCENTAGE OF NEURONS
AZURE B-RNA (ABSORBANCY UNITS)
(LOW, MODERATE, HIGH RNA NEURONS)

A-1174


THALAMIC NEURONAL RNA RESPONSES IN SOMAN TOXICATED RATS
NUCLEUS RETICULARIS
VENTROBASAL COMPLEX
CONTROL
0.5 LD50
0.9 LD50
1.5 LD50
PERCENTAGE OF NEURONS
60
40
20
15
16
12
13
29
12
4
20
23
4
33
1
3
38
61
0
60 80 100
40 80 120
AZURE B-RNA (ABSORBANCY UNITS)
(LOW, MODERATE, HIGH RNA NEURONS)


SUBSTANTIA NIGRA NEURONAL RNA RESPONSES IN SOMAN TOXICATED RATS
PARS COMPACTA
PARS RETICULATA
CONTROL
0.5 LD50
0.9 LD50
1.5 LD50
PERCENTAGE OF NEURONS
60
40
20
14
19
20
16
22
7
28
20
31
3
10
37
69
1
23
26
60 80 100
40 80 120
AZURE B-RNA (ABSORBANCY UNITS)
(LOW, MODERATE, HIGH RNA NEURONS)

A-1176



RETICULAR FORMATION NUCLEI NEURONAL RNA RESPONSES IN SOMAN TOXICATED RATS
NUCLEUS CUNEIFORMIS
VENTROTEGMENTAL NUCLEUS
CONTROL
18
17
19
10
0.5 LD50
1
30
24
17
0.9 LD50
38
5
56
4
1.5 LD50
76
0
77
0
60
40
20
PERCENTAGE OF NEURONS
80 100 120
AZURE B-RNA (ABSORBANCY UNITS)
(LOW, MODERATE, HIGH RNA NEURONS)

A-1177

FIGURE 7

SUMMARY OF RNA RESPONSE PATTERNS WITH LOW AND HIGH SOMAN DOSAGES

BRAIN REGION	LOW	HIGH
THALAMUS		
VENTROBASAL NUCLEAR COMPLEX*	↑	↓
NUCLEUS RETICULARIS+	↓	↑
LATERAL NUCLEUS	—	↓
RETICULAR FORMATION		
NUCLEUS CUNEIFORMIS*	↑	↓
VENTROTEGMENTAL NUCLEUS*	—	↓
SUBSTANTIA NIGRA		
PARS COMPACTA	↓	↓
PARS RETICULATA	—	↑ or —
STRIATUM	↓	↓
CEREBRAL CORTEX		
SENORIMOTOR	↓	↓
PYRIFORM	—	↓

* BRAIN SITES CONTAINING PREDOMINANTLY ACH-EXCITED NEURONS

\+ BRAIN REGION WHERE ACH ACTS AS INHIBITORY NEUROTRANSMITTER

↑ INDICATES RNA ELEVATION

↓ INDICATES RNA DEPRESSION

— INDICATES NO RNA CHANGE

CONCLUSIONS

1) AN ASYMPTOMATIC (0.5 LD_{50}) SOMAN CHALLENGE FACILITATES SUBCORTICAL CHOLINERGIC TRANSMISSION AS EVIDENCED BY NEURONAL RNA ACTIVATION WITHIN ACH-EXCITED NUCLEI, I.E., VBC AND NC, AND RNA DEPRESSION WITHIN THE ACH-INHIBITED NR.

2) WITH HIGH DOSAGES OF SOMAN (0.9 AND 1.5 LD_{50}), DIRECT OPPOSITE RNA RESPONSE PATTERNS WERE DETECTED WITHIN THESE NUCLEI, SUGGESTING IMPAIRED CHOLINERGIC NEUROTRANSMISSION OR DISRUPTION OF THE FUNCTIONAL INTEGRITY OF ASCENDING ACH-MEDIATED PATHWAYS (MRF) AND THALAMIC RELAY NUCLEI.

3) IMPAIRED CHOLINERGIC RESPONSIVENESS OF SUBCORTICAL COMPARTMENTS MAY RESULT FROM MUSCARINIC RECEPTOR DOWN-REGULATION OR BLOCKADE WHICH COULD IN TURN PRODUCE A FOREBRAIN "DEAFFERENTATION" AND CEREBROCORTICAL RNA LOSS.

4) RNA DEPLETION IN CHOLINERGIC COMPARTMENTS AND DOPAMINERGIC BRAIN SITES, I.E., SUBSTANTIA NIGRA PARS COMPACTA, SUGGESTS THAT SOMAN LETHALITY IS NOT ATTRIBUTABLE TO CNS CHOLINERGIC HYPEREXCITATION BUT RATHER TO A BREAKDOWN OF EFFECTIVE EXCITATORY-INHIBITORY NEUROTRANSMISSION WITH CHOLINERGIC AND NONCHOLINERGIC MECHANISMS BEING EQUALLY IMPORTANT.

LOCAL CHOLINERGIC MECHANISMS MEDIATE THE CEREBRAL CORTICAL VASODILATION ELICITED BY ELECTRICAL STIMULATION OF THE FASTIGIAL NUCLEUS IN RAT

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INTRODUCTION

ELECTRICAL STIMULATION OF THE FASTIGIAL NUCLEUS (FN) OF CEREBELLUM INCREASES REGIONAL CEREBRAL BLOOD FLOW (rCBF) IN RATS GLOBALLY AND MAXIMALLY IN THE CEREBRAL CORTEX (UP TO 250% OF CONTROL; NAKAI ET AL., AM. J. PHYSIOL. 243: H226, 1982). THE CORTICAL INCREASES IN rCBF OCCUR INDEPENDENT OF CHANGES IN REGIONAL GUCLOSE UTILIZATION (NAKAI ET AL., BRAIN RES. 260: 35, 1983). UNILATERAL ELECTROLYTIC LESIONS OF THE BASAL FOREBRAIN, THE MAJOR SOURCE OF CHOLINERGIC INPUT TO THE CEREBRAL CORTEX, IPSILATERALLY ABOLISHES THE INCREASE IN rCBF ELICITED BY FN STIMULATION (IADECOLA ET AL., BRAIN RES. 279: 41, 1983).

WE SOUGHT TO DETERMINE WHETHER THE CORTICAL CEREBROVASODILATION ELICITED BY FN STIMULATION IS MEDIATED BY CHOLINERGIC MECHANISMS.

HYPOTHESIS:

CHOLINERGIC MECHANISMS GOVERN THE CORTICAL CEREBROVASODILATION ELICITED BY ELECTRICAL STIMULATION OF FASTIGIAL NUCLEUS (FN) OF THE CEREBELLUM IN RAT?

METHODS

A. SURGICAL PROCEDURES AND CBF MEASUREMENT

1. ANESTHESIA WAS INDUCED BY HALOTHANE (2-3%) AND MAINTAINED BY CHLORALOSE (40 mg/kg, s.c.); CATHETERS WERE PLACED IN THE TRACHEA AND FEMORAL ARTERIES AND VEINS; PARALYSIS WAS INDUCED BY TUBOCURARINE, AND THE ANIMALS WERE ARTIFICIALLY VENTILATED WITH 100% O_2.

2. A BURR HOLE WAS DRILLED IN THE SKULL OVERLYING THE PARIETAL CORTEX, DURA REFLECTED, AND THE DEVICE USED TO APPLY DRUGS TO THE BRAIN SURFACE WAS STEREOTAXICALLY POSITIONED (SEE FIG. 2).

3. CORTICAL TEMPERATURE, BODY TEMPERATURE, ARTERIAL BLOOD GASES, ARTERIAL PRESSURE AND HEART RATE WERE MONITERED AND MAINTAINED IN PHYSIOLOGICAL RANGES.

4. THE FASTIGIAL NUCLEUS (FN) OF THE CEREBELLUM WAS ELECTRICALLY STIMULATED WITH INTERMITTENT TRAINS OF PULSES (50 Hz, 0.5 MSEC, 1 SEC ON/1 SEC OFF) AT 5 TIMES THE THRESHOLD CURRENT FOR A PRESSOR RESPONSE.

5. MEAN ARTERIAL PRESSURE (MAP) WAS MAINTAINED WITHIN THE AUTOREGULATED RANGE OF CBF (80-150 mmHg) DURING FN STIMULATION.

6. CBF WAS MEASURED REGIONALLY USING THE ^{14}C-IODOANTIPYRINE TECHNIQUE BY DISSECTION (OHNO ET AL., STROKE 10:62, 1979).

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PROTOCOL USED TO MEASURE CEREBRAL BLOOD FLOW IN THE RAT FOLLOWING ELECTRICAL STIMULATION OF THE FASTIGIAL NUCLEUS

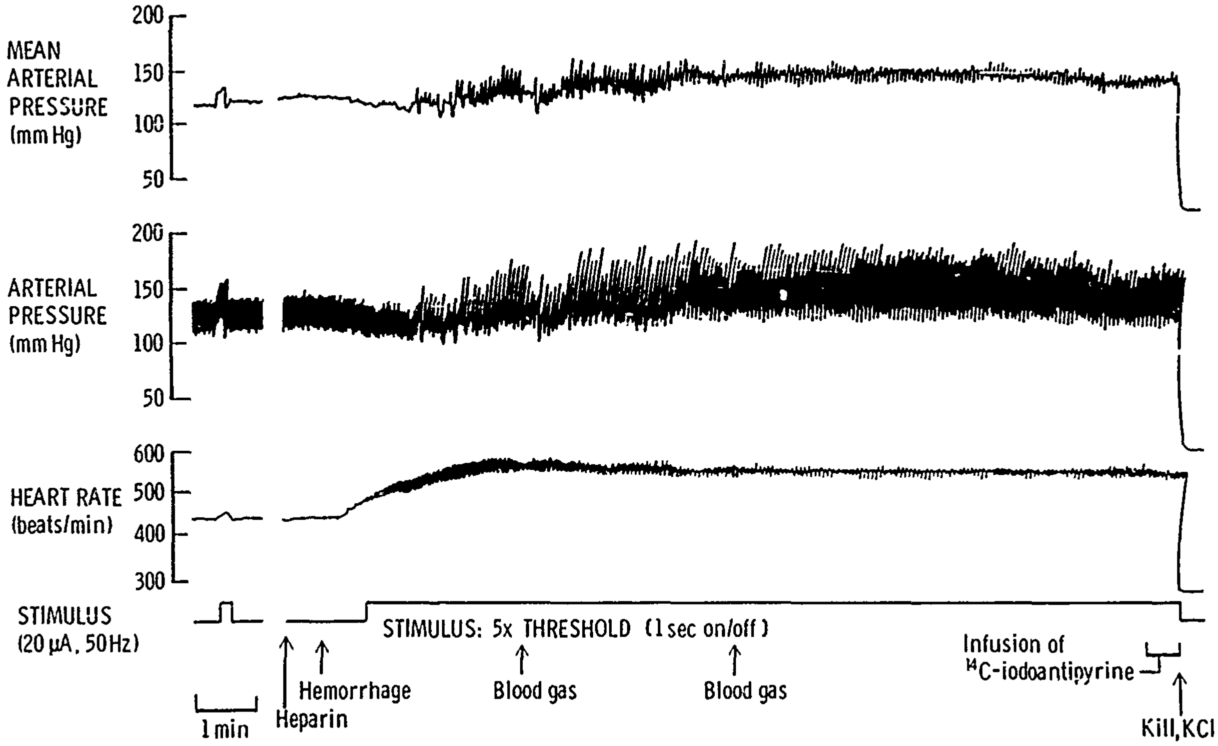


Table I. Mean arterial pressure (MAP), pH and arterial blood gases in rats examined

	UNSTIMULATED		FN-STIMULATION		HYPERCARBIA
	VEHICLE (N=6)	ATROPINE (N=4)	VEHICLE (N=6)	ATROPINE (N=5)	ATROPINE (N=5)
MAP (mmHg)	124 ± 6	136 ± 4	134 ± 2	130 ± 5	110 ± 6
pO_2 (mmHg)	429 ± 19	431 ± 20	407 ± 13	409 ± 21	408 ± 30
pCO_2 (mmHg)	35.7 ± 0.5	34.9 ± 1.0	36.8 ± 0.6	36.6 ± 0.9	59.0 ± 1.4*
pH	7.41 ± 0.02	7.41 ± 0.01	7.36 ± 0.02*	7.32 ± 0.01*	7.24 ± 0.01*

Values represent means ± S.E.M.; animals are anesthetized, paralyzed and artificially respirated;

* $p < 0.05$, significantly different from vehicle control.

B. CORTICAL APPLICATION TECHNIQUE

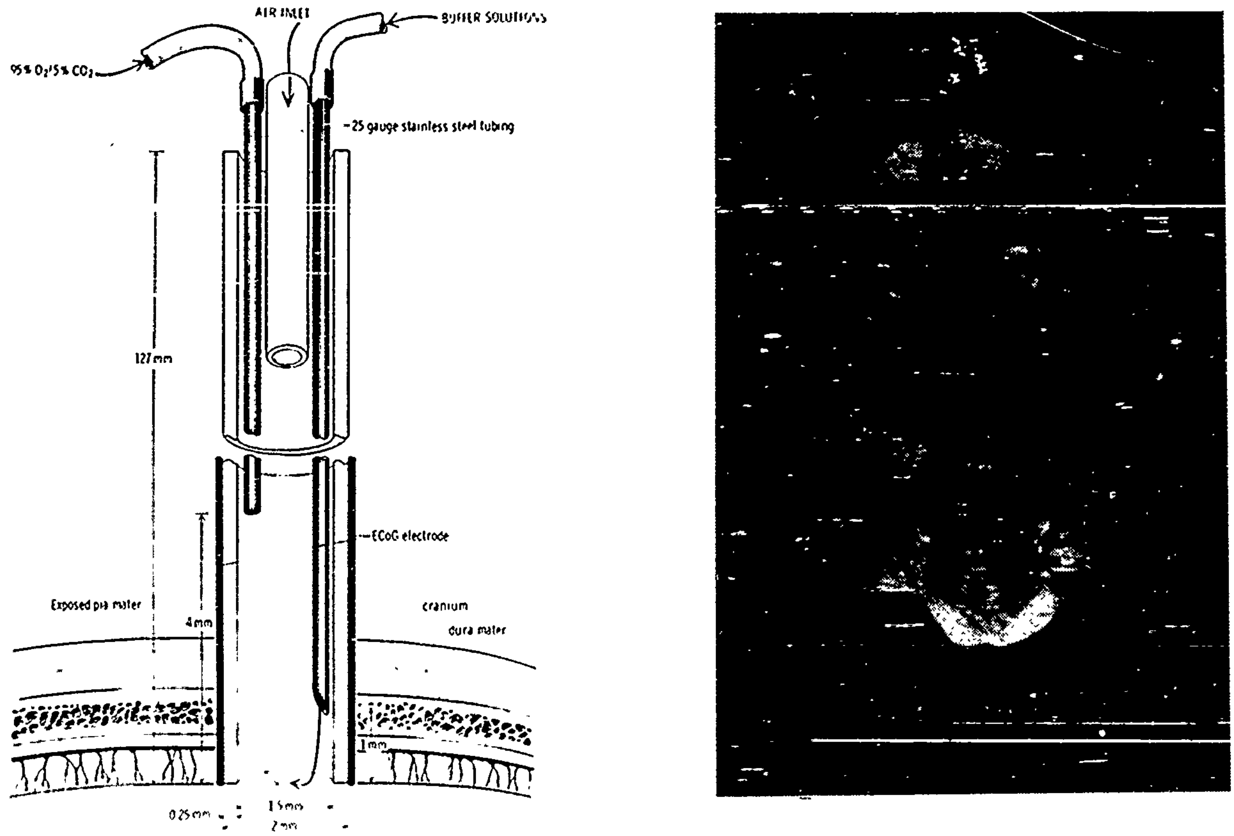


FIG. 2 PANEL A: A SCHEMATIC OF THE DEVICE THAT WAS USED TO APPLY DRUGS TO THE SURFACE OF THE CFREBRAL CORTEX. PANEL B: INTRODUCTION OF THE DYE FAST GREEN INTO THE APPLICATOR INDICATES THE RESTRICTED AREA OF THE BRAIN WHERE THE DRUGS WERE APPLIED.

Table II. pH, pCO_2 and pO_2 of buffer applied to the parietal cortex of rats with and without FN-stimulation or hypercarbia.[a,b,c]

			UNSTIMULATED	FN-STIMULATION		HYPERCARBIA
			VEHICLE (N=6)	VEHICLE (N=6)	ATROPINE[d] (N=5)	ATROPINE[d] (N=5)
pH		R	7.40 ± 0.05	7.41 ± 0.09	7.54 ± 0.05	7.63 ± 0.09
		L	7.46 ± 0.80	7.36 ± 0.07	7.39 ± 0.07	7.58 ± 0.09
pCO_2	(mmHg)	R	32.7 ± 4.2	32.4 ± 2.7	25.8 ± 6.0	21.8 ± 3.1*
		L	33.7 ± 3.8	32.4 ± 2.7	32.9 ± 5.1	20.5 ± 2.0*
pO_2	(mmHg)	R	428 ± 77	298 ± 48	394 ± 67	373 ± 72
		L	425 ± 78	290 ± 45	463 ± 77	384 ± 50

(a) Values are means ± S.E.M.; * $p < 0.05$, significantly different from corresponding vehicle, unstimulated control (ANOVA).

(b) No right to left differences with any treatment ($p > 0.05$; paired t-test).

(c) Kreb's-bicarbonate buffer solutions were bubbled with 95% O_2: 5% CO_2, the above values determined and the solution applied to the parietal cortex. Solutions were continuously bubbled with 95% O_2: 5% CO_2 following application to the cortex.

(d) Atropine sulfate (100 uM) applied to right parietal cortex.

C. MICROVESSEL PREPARATION

A.

MICROVESSEL AND SYNAPTOSOME ISOLATION PROCEDURE

1. Rapidly remove 100-200 mg tissue
2. Homogenize in 10 vol. 0.32M sucrose (4^{o}C); 7-10 strokes
3. Centrifuge at 1000 x g for 10 min.

MICROVESSELS (Pellet - P_1)

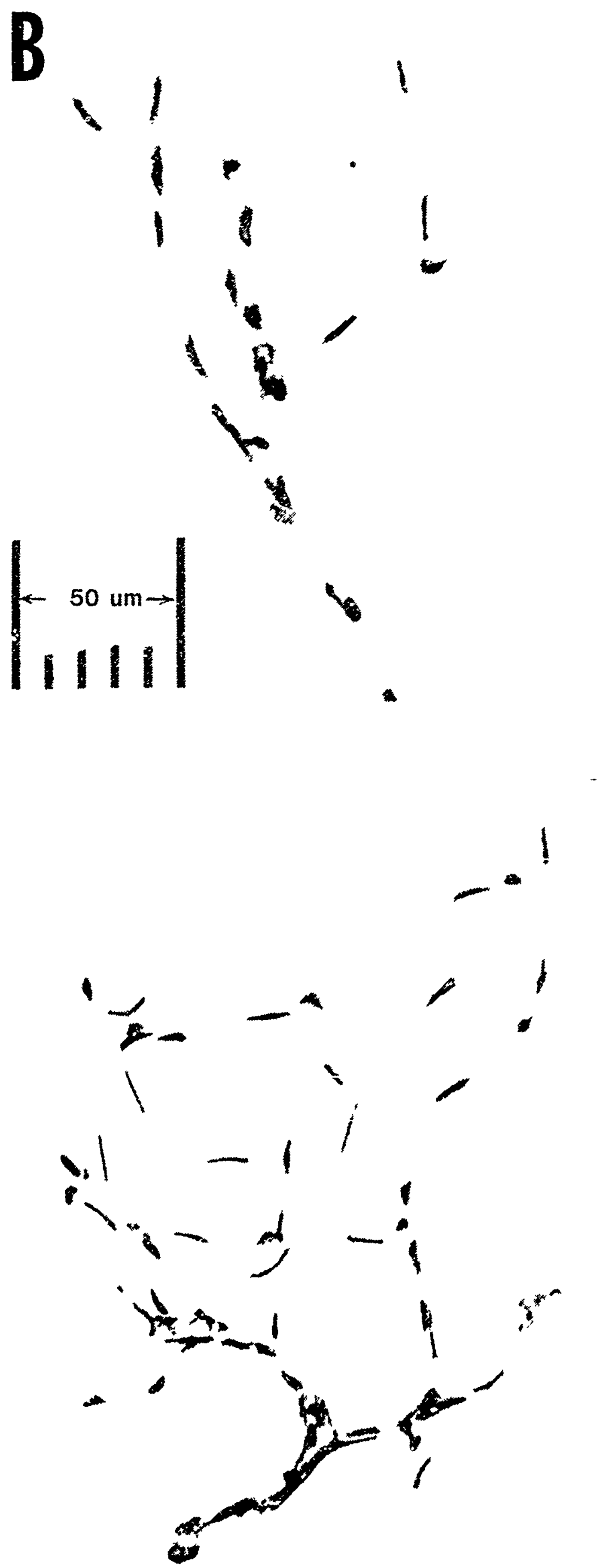
1. Resuspend P_1 in 0.5 ml 0.25M sucrose (4^{o}C) and layer on discontinuous sucrose gradient (0.5 ml, P_1, top; 2.0 ml 1M sucrose, middle; 3.0 ml 1.5M sucrose, bottom)
2. Centrifuge at 65,000 x g for 45 min.
3. Discard supernatant; resuspend pellet in 10 vol. Kreb's-bicarbonate buffer.
   —This is referred to as the microvessel fraction.

SYNAPTOSOMES (Supernatant - S_1)

1. Centrifuge at 27,000 xg for 20 min.
2. Discard supernatant; resuspend P_2-pellet in 10 vol. Kreb's-bicarbonate buffer.
   —This is referred to as the synaptosomal fraction.

FIG. 3 PANEL A: THE PROTOCOL USED FOR THE ISOLATION OF SYNAPTOSOMES AND CEREBRAL CORTICAL MICROVESSELS. WITH SOME MODIFICATION, THE FRACTIONS WERE ISOLATED ACCORDING TO THE PROCEDURE OF REINHARD AND CO-WORKERS (SCIENCE 106:85, 1979). PANEL B: LIGHT MICROGRAPH OF BRAIN MICROVESSEL FRACTION ISOLATED FROM RAT CEREBRAL CORTEX. VESSELS WERE STAINED WITH METHYLENE BLUE.

A-1186



A-1187

A. DOES ATROPINE BLOCK THE FN-ELICITED INCREASE IN rCBF?

1. DOES SYSTEMIC ATROPINE ADMINISTRATION BLOCK THE VASODILATOR RESPONSE?

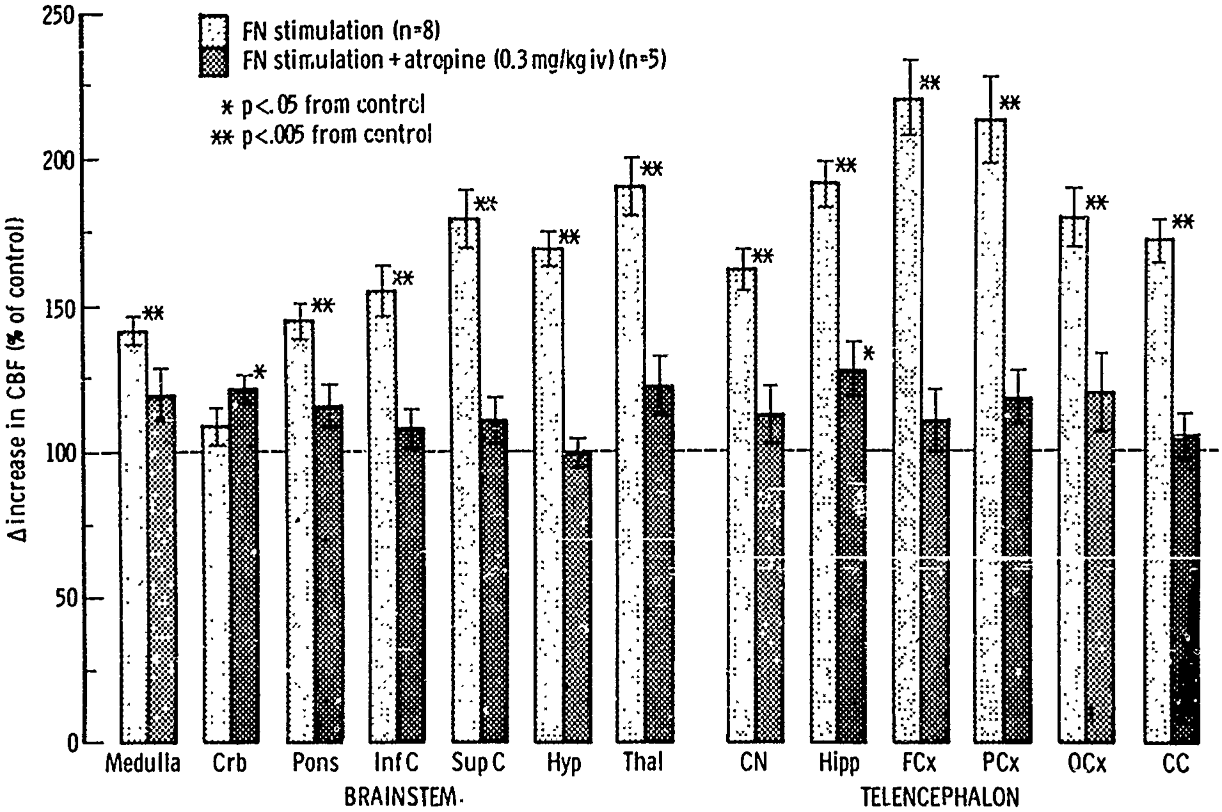


FIG. 4: ATROPINE WAS GIVEN I.V. 15 MIN. PRIOR TO FN STIMULATION. ABBREVIATIONS USED ABOVE FROM LEFT TO RIGHT: Crb, CEREBELLUM; InfC, INFERIOR COLLICULUS; SupC, SUPERIOR COLLICULUS; Hyp, HYPOTHALAMUS; Thal, THALAMUS; CN, CAUDATE NUCLEUS; Hipp, HIPPOCAMPUS; FCx, FRONTAL CORTEX; PCx, PARIETAL CORTEX; OCx, OCCIPITAL CORTEX; AND CC, CORPUS CALLOSUM.

WITH SYSTEMIC ATROPINE THE INCREASES IN rCBF ARE NEARLY ABOLISHED IN MOST OF THE REGIONS STUDIED.

2. DOES LOCAL ATROPINE APPLICATION TO THE CEREBRAL CORTEX BLOCK THE CORTICAL INCREASE IN rCBF?

EFFECT OF ATROPINE SULFATE (100μM) APPLIED TO THE RIGHT PARIETAL CORTEX ON INCREASES IN rCBF ELICITED BY ELECTRICAL STIMULATION OF THE FASTIGIAL NUCLEUS

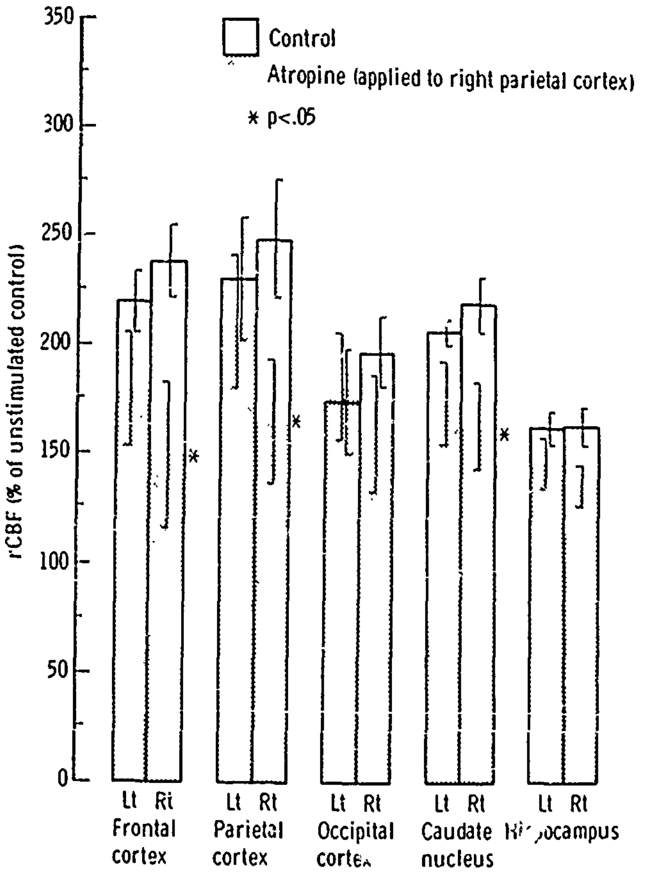


FIG. 5: ATROPINE (100 uM) WAS APPLIED TO THE CORTEX 10 MIN. PRIOR TO FN STIMULATION. VALUES ARE MEANS ± S.E.M.; N=5-3; * p ∠ 0.05 ALL VALUES ARE SIGNIFICANTLY ELEVATED ABOVE UNSTIMULATED CONTROLS, p∠0.05.

WITH LOCAL ATROPINE PRETREATMENT THE INCREASES IN rCBF ARE MARKEDLY ATTENUATED IPSILATERAL TO THE SIDE OF APPLICATION.

3. IS THE BLOCKING EFFECT OF ATROPINE SPECIFIC TO THE FN-ELICITED CORTICAL VASODILATION?

EFFECT OF ATROPINE SULFATE (100µM) APPLIED TO THE RIGHT PARIETAL CORTEX ON CO_2 ELICITED INCREASES IN rCBF

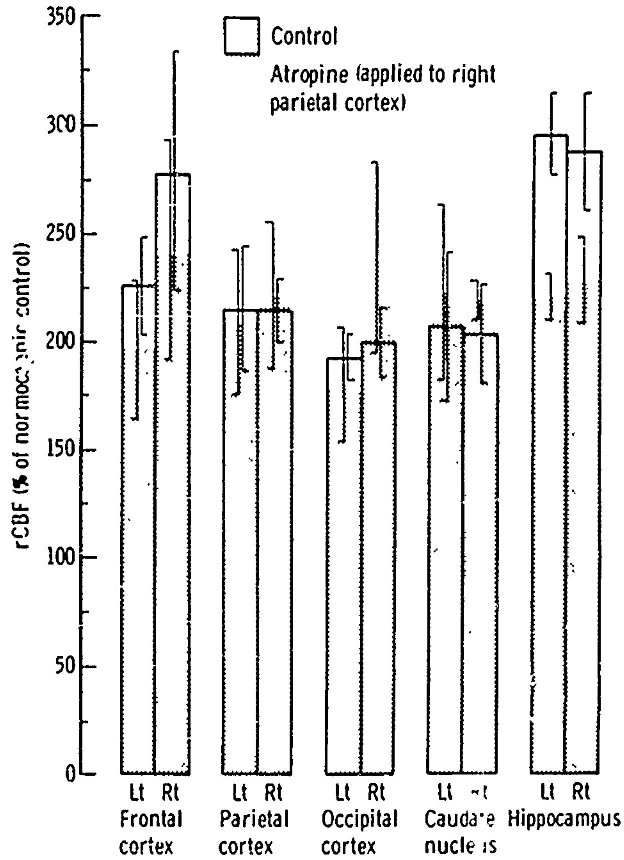


FIG. 6: ATROPINE (100 uM) WAS APPLIED TO THE CORTEX 10 MIN. PRIOR TO ONSET OF VENTILATION WITH 5% CO_2/95% O_2. THE CONTROL VALUES WERE OBTAINED FROM UNOPERATED (CLOSED SKULL), HYPERCAPNIC ANIMALS ($pCO_2 = 60.4 \pm 1.1$, n=5). VALUES ARE MEANS + S.E.M.; ALL VALUES ARE SIGNIFICANTLY ELEVATED ABOVE NORMOCAPNIC CONTROLS, $p < 0.05$.

ATROPINE DID NOT ATTENUATE THE VASODILATION ELICITED BY HYPERCARBIA.

Table III. Effects of topical application of atropine sulfate on cerebral blood flow (rCBF) in rats with and without stimulation of fastigial nucleus or hypercarbia.

Brain Region		rCBF (ml/100g min) ± S.E.M.				
		UNSTIMULATED		FN-STIMULATION		HYPERCARBIA
		VEHICLE[a] (N=6)	ATROPINE[a,c] (N=4)	VEHICLE[a,d] (N=6)	ATROPINE[c,d] (N=5)	ATROPINE[a,c,d] (N=5)
Frontal Cortex	R	79 ± 7	91 ± 13	213 ± 15[b]	133 ± 30*	200 ± 42
	L	93 ± 12	100 ± 13	193 ± 13	160 ± 23	188 ± 31
Parietal Cortex	R	70 ± 6	85 ± 9	195 ± 21[b]	129 ± 22*	168 ± 26
	L	82 ± 12	83 ± 7	181 ± 22	165 ± 24	171 ± 28
Occipital Cortex	R	72 ± 7	85 ± 9	161 ± 13[b]	130 ± 22	184 ± 34
	L	86 ± 12	88 ± 6	142 ± 20	148 ± 20	155 ± 25
Caudate Nucleus	R	75 ± 6	85 ± 10	172 ± 10	128 ± 16*	174 ± 7
	L	75 ± 6	84 ± 7	162 ± 5	137 ± 15	176 ± 7
Hippocampus	R	74 ± 6	78 ± 7	124 ± 7	103 ± 7	172 ± 15
	L	78 ± 8	72 ± 3	123 ± 6	111 ± 9	168 ± [illegible]

(a) Differences between right and left sides not significant ($p \geq 0.05$; paired t-test)
(b) Values significantly increased over contralateral side ($p < 0.05$; paired t-test)
(c) Atropine sulfate (100 uM) applied to only right parietal cortex
(d) All values significantly increased above unstimulated vehicle control ($p < 0.05$; ANOVA).
* $p < 0.05$, values significantly decreased from corresponding vehicle control, FN-stimulated group ($p < 0.05$; ANOVA)

B. ARE CHOLINERGIC MARKERS ASSOCIATED WITH CEREBROCORTICAL MICROVESSELS?

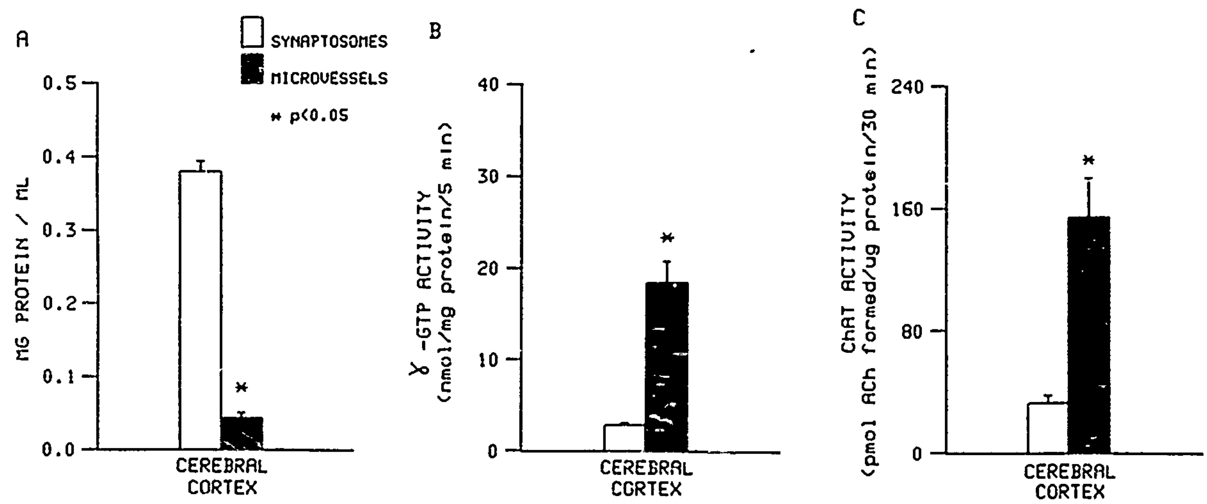


FIG. 7: THE RELATIVE AMOUNTS OF PROTEIN AND ENZYMATIC ACTIVITIES ASSOCIATED WITH CEREBRAL CORTICAL SYNAPTOSOMES AND MICROVESSELS. PANEL A: THE AMOUNT OF PROTEIN PRESENT IN EACH ISOLATED FRACTION. PANEL B: THE ACTIVITY OF GAMMA-GLUTAMYLTRANSPEPTIDASE (γ-GTP) WITHIN SYNAPTOSOMES AND MICROVESSELS. γ-GTP IS A SPECIFIC MARKER OF CEREBRAL CORTICAL ENDOTHELIAL CELLS AND WAS QUANTIFIED BY THE METHOD OF ORLOWSKY AND MEISTER (J. BIOL. CHEM. 240-338, 1965). PANEL C: THE ACTIVITY OF CHOLINE ACETYLTRANSFERASE (ChAT), THE SYNTHESIZING ENZYME OF ACETYLCHOLINE, WITHIN SYNAPTOSOMES AND ENRICHED MICROVESSELS. ChAT ACTIVITY WAS MEASURED BY THE METHOD OF FONNUM (J. NEUROCHEM. 24:407 1975). VALUES ARE MEANS $\pm$ S.E.M.; N=3; * $p < 0.05$.

THE ACTIVITY OF ChAT IS GREATER IN THE MICROVESSEL FRACTION.

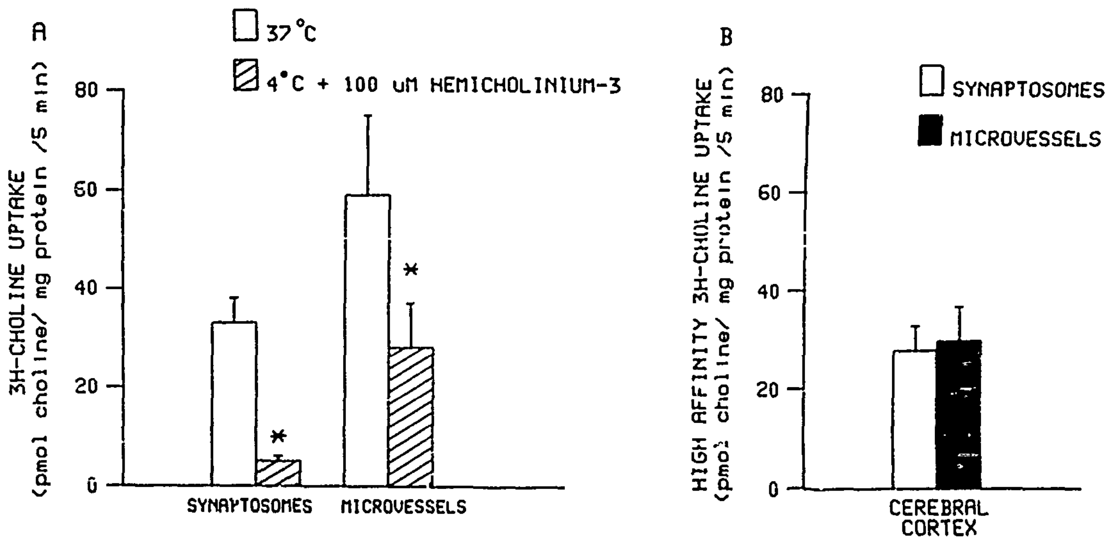


FIG. 8: PANEL A: THE COMBINED EFFECT OF LOW TEMPERATURE AND HEMICHOLINIUM-3, AN INHIBITOR OF THE HIGH-AFFINITY UPTAKE PROCESS, ON THE UPTAKE OF ^{3}H-CHOLINE INTO RAT CEREBRAL CORTICAL SYNAPTOSOMES AND MICROVESSELS. PANEL B: THE FRACTION OF THE TOTAL ^{3}H-CHOLINE UPTAKE WHICH OCCURS VIA THE TEMPERATURE-DEPENDENT, HIGH-AFFINITY UPTAKE PROCESS. VALUES ARE MEANS $\pm$ S.E.M.; N=3; * $p < 0.05$.

THE HIGH-AFFINITY UPTAKE PROCESS FOR CHOLINE INTO CEREBRAL CORTICAL SYNAPTOSOMES AND MICROVESSELS DOES NOT QUANTITATIVELY DIFFER BETWEEN THESE TISSUE FRACTIONS.

SUMMARY

1. **SYSTEMIC ATROPINE** ADMINISTRATION PREVENTS THE INCREASES IN rCBF ELICITED IN MOST OF THE BRAIN REGIONS STUDIED.

2. **LOCAL ATROPINE** APPLICATION TO THE PARIETAL CORTEX MARKEDLY ATTENUATES THE INCREASE IN CORTICAL rCBF ELICITED BY FN STIMULATION, BUT DOES NOT ALTER RESTING CORTICAL rCBF.

3. IN CONTRAST, **LOCAL ATROPINE** APPLICATION TO THE PARIETAL CORTEX DOES NOT ATTENUATE THE CORTICAL VASODILATION ELICITED BY HYPERCARBIA.

4. **CHOLINERGIC MARKERS**, SUCH AS CHOLINE ACETYLTRANSFERASE AND HIGH-AFFINITY CHOLINE UPTAKE ACTIVITY, ARE FOUND IN ENRICHED FRACTIONS OF RAT CORTICAL MICROVESSELS.

CONCLUSIONS

1. CORTICAL CEREBROVASODILATION ELICITED BY FN-STIMULATION, BUT NOT BY CO_2, IS IN LARGE PART MEDIATED BY CORTICAL MUSCARINIC CHOLINERGIC RECEPTORS.

2. IT IS LIKELY THAT RELEASE OF ACETYLCHOLINE AT THE LEVEL OF THE CORTICAL MICROVASCULATURE IS INVOLVED IN THIS RESPONSE, SINCE ACETYLCHOLINE CAN BE SYNTHESIZED IN ELEMENTS CONTAINED WITHIN OR IN CLOSE APPOSITION TO CORTICAL MICROVESSELS.

(This work supported in part by the U.S. Army Medical Research and Development Command under Contract DAMD-17-84-C-4185.)

PHYSOSTIGMINE TOXICITY: ACUTE AND DELAYED EFFECTS ON NEUROMUSCULAR JUNCTIONS

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Adult albino rats were administered single subcutaneous injections of physostigmine at 0.001 to 1.1 LD_{50} (LD_{50}=0.75 mg/kg). Samples of diluted whole blood were obtained immediately before injection and at ½ hour, 1, 7, 14, 28, and 56 days post injection (PI). Rats were fixed by whole body perfusion at the same intervals PI and examined by thin section electron microscopy. Blood cholinesterase inhibition levels were correlated with changes in muscle and nerve ultrastructure and physiology. Enzyme inhibitions of ≃10% at 0.001 LD_{50}, ≃30% at 0.01 LD_{50}, and ≃67% at 0.1 LD_{50} were measured ½ hour PI, but major destructive effects were not observed in EDL, soleus, or diaphragm myofibers. Thus, enzyme inhibitions of < 67% were associated with no major changes in myofiber ultrastructure. However, ½ hour after single injections of 0.8 to 1.1 LD_{50}, enzyme inhibitions of 80%-98% were measured, and all neuromuscular junctions of the constantly-used diaphragm and soleus myofibers exhibited supercontraction of sarcomeres in the subjunctional sarcoplasm. Often, Z bands were missing, free thick and thin filaments were present in disorganized masses, and a mixed population of "frothy" and grossly distended mitochondria were observed disrupting the subjunctional sarcoplasm. However, EDL muscles from the same rats were much less affected. Some fibers exhibited "blistered" or "frothy" mitochondria in the immediate subsynaptic regions, while many fibers exhibited little or no changes in sarcomere ultrastructure. At 24 hours PI, the destructive effects observed in diaphragm and soleus fibers were partially reversed, substantially reversed by 7 days, and virtually indetectable by 14 days. In contrast, at 24 hours PI, EDL fibers exhibited increased myofiber damage and increased "frothy" and partially distended mitochondria. This delay in the appearance of destructive alterations in EDL fibers may be related to the resumption of EDL muscle activity during the ensuing 6 to 12 hour period of low esterase activity. Since these same rats had recovered their respiratory capabilities and, ultrastructurally, exhibited partially repaired diaphragm myofibers, the delayed damage to EDL fibers is presumed to be related to the resumption of voluntary myofiber activity associated with walking and grooming. By 7 days PI, junctional folds in some diaphragm and soleus myofibers were devoid of attached nerve terminals. By 14 days PI, nerve processes of very small diameter were observed, some of which were <u>not</u> embedded within a primary synaptic cleft. This suggests a process of partial denervation and reinnervation via small collateral "sprouts." During this same period, strong variations in enzyme activity (from 30% to 200% of normal activity for the same rat) were observed up to 8 weeks after single high dose injections, while similar large-scale changes were not observed in "control" or "low dose" animals. Finally, physiological effects on skeletal muscle contractility, as demonstrated by potentiation of EDL twitch tensions, were observed 20-50 minutes PI at $\geq$ 0.05 LD_{50} but not at $\leq$ 0.025 LD_{50}. These data indicate that a) physostigmine-induced <u>supercontraction</u>, as well as other changes in myofiber ultrastructure, are <u>dose dependent</u>, with severe damage occurring above 70% enzyme inhibition; b) <u>delayed reactions</u> to anticholinesterase exposure include <u>partial denervation</u> and <u>reinnervation phenomena</u>, as well as long-term fluctuations in blood cholinesterase activity; c) the <u>time-course</u> of physostigmine-induced denervation and reinnervation is relatively rapid, perhaps within 1 to 2 weeks of a high dose exposure to physostigmine; and d) <u>muscle use</u> may be an important factor in <u>drug toxicity, habituation, and reversibility</u>.

This work supported in part by the US Army Medical Research and Development Command under Contract DAMD-17-84-C-4010.

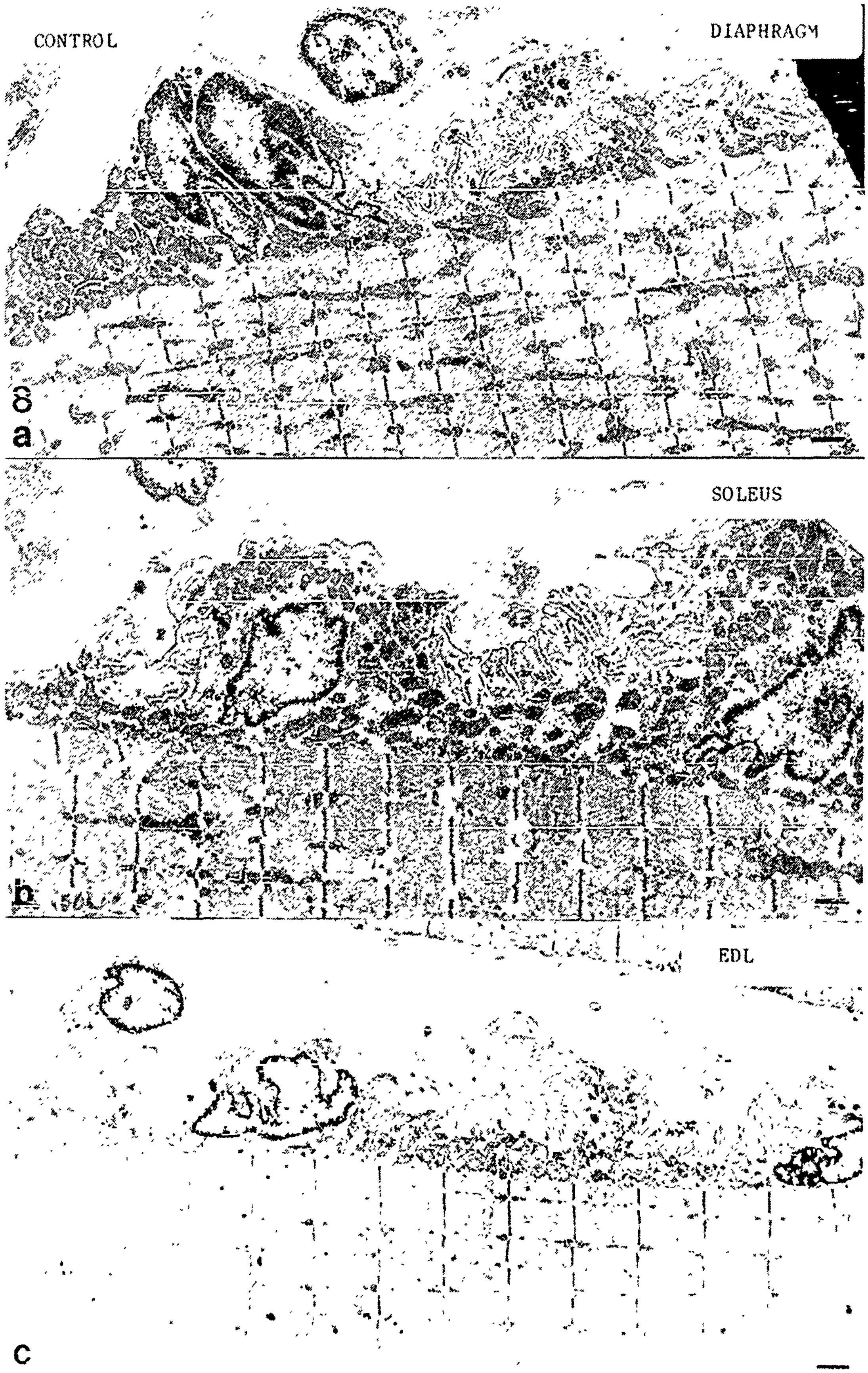


Fig. 8. Side-by-side comparison of neuromuscular junctions from "control" diaphragm (Fig. 8a), soleus (Fig. 8b0, and EDL (Fig. 8c) muscles 30 minutes after sham injection.

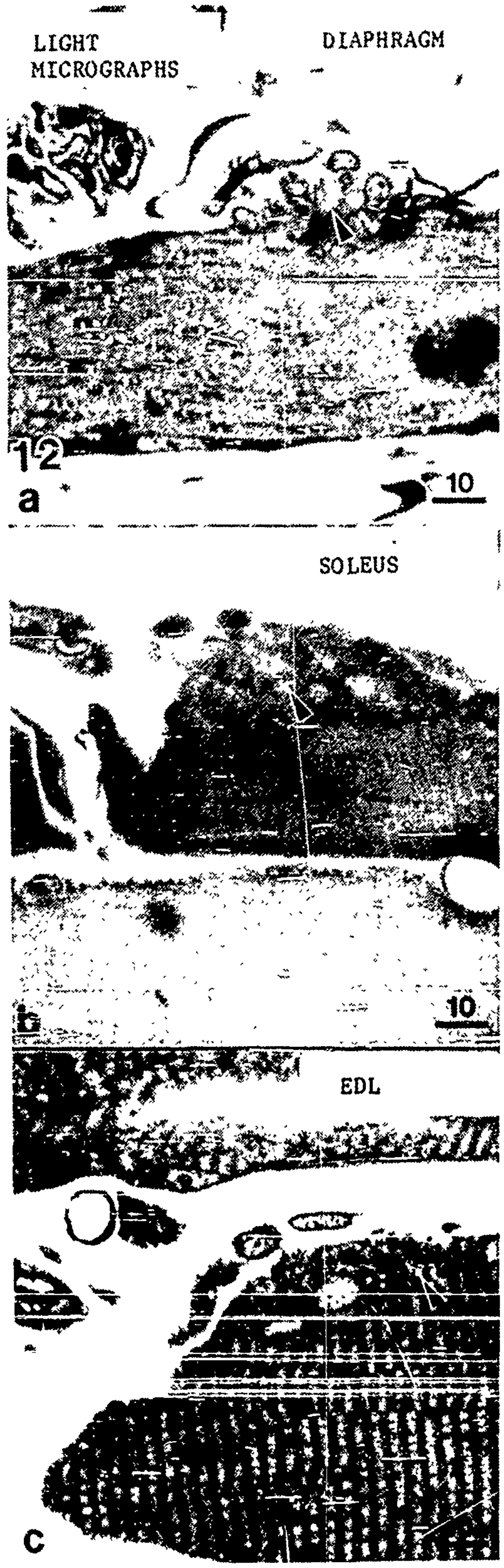


Fig. 12. Side-by-side comparison of light micrographs showing the effects of an acute high dose of physostigmine (0.8-1.1 LD_{50}) on neuromuscular junctions of diaphragm (Fig. 12a), soleus (Fig. 12b), and EDL (Fig. 12c) 30 minutes PI. Supercontraction and disruption of myofibrils in the subjunctional sarcoplasm of diaphragm and soleus fibers is evident, as in mitochondrial swelling (arrowheads) in all three fiber types. However, at light microscopic magnification and resolution, endplates are relatively difficult to find/identify, and resolution of pathological details is not possible.

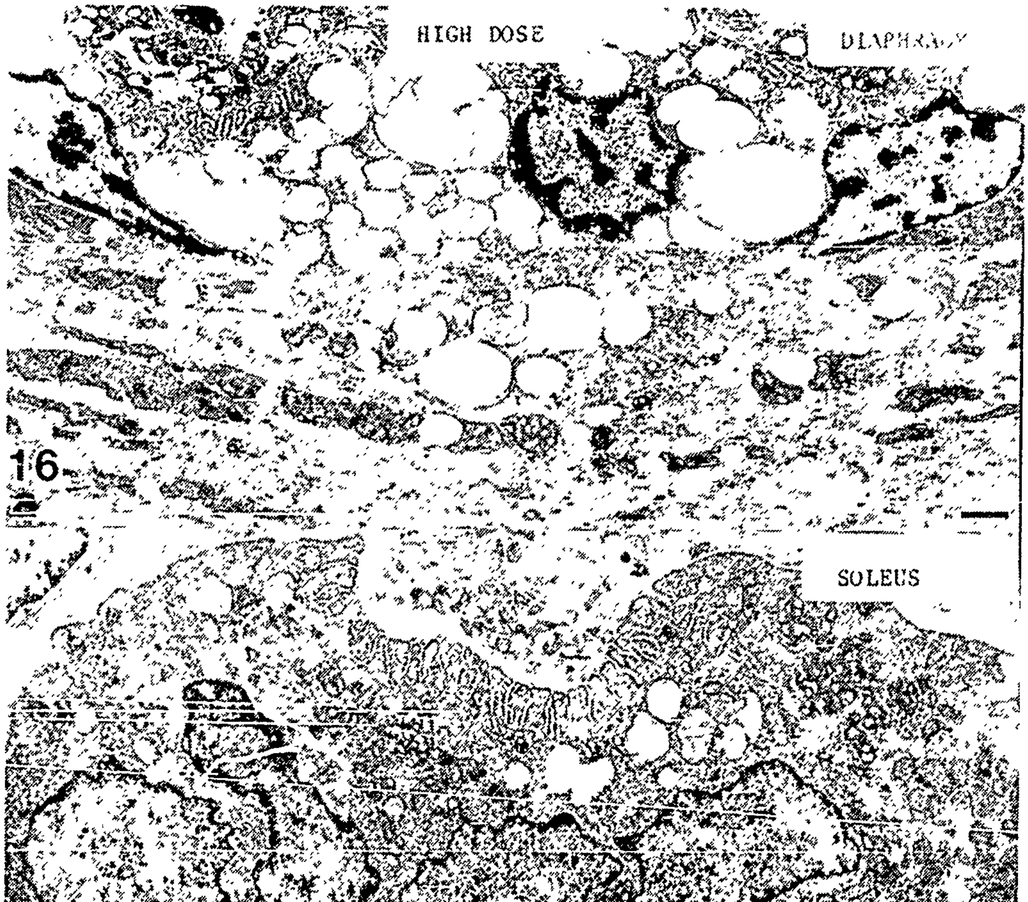


Fig. 16. Side-by-side comparison of the effects of an acute high dose of physostigmine (0.8-1.1 LD_{50}) on neuromuscular junctions of diaphragm (Fig. 16a), soleus (Fig. 16b), and EDL (Fig. 16c) 30 minutes PI. These three electron micrographs were taken from the same specimens used for light microscopy (Figs. 12a-c). Structural alterations may be identified more quickly and visualized at much greater resolution by electron microscopy than by light microscopy.

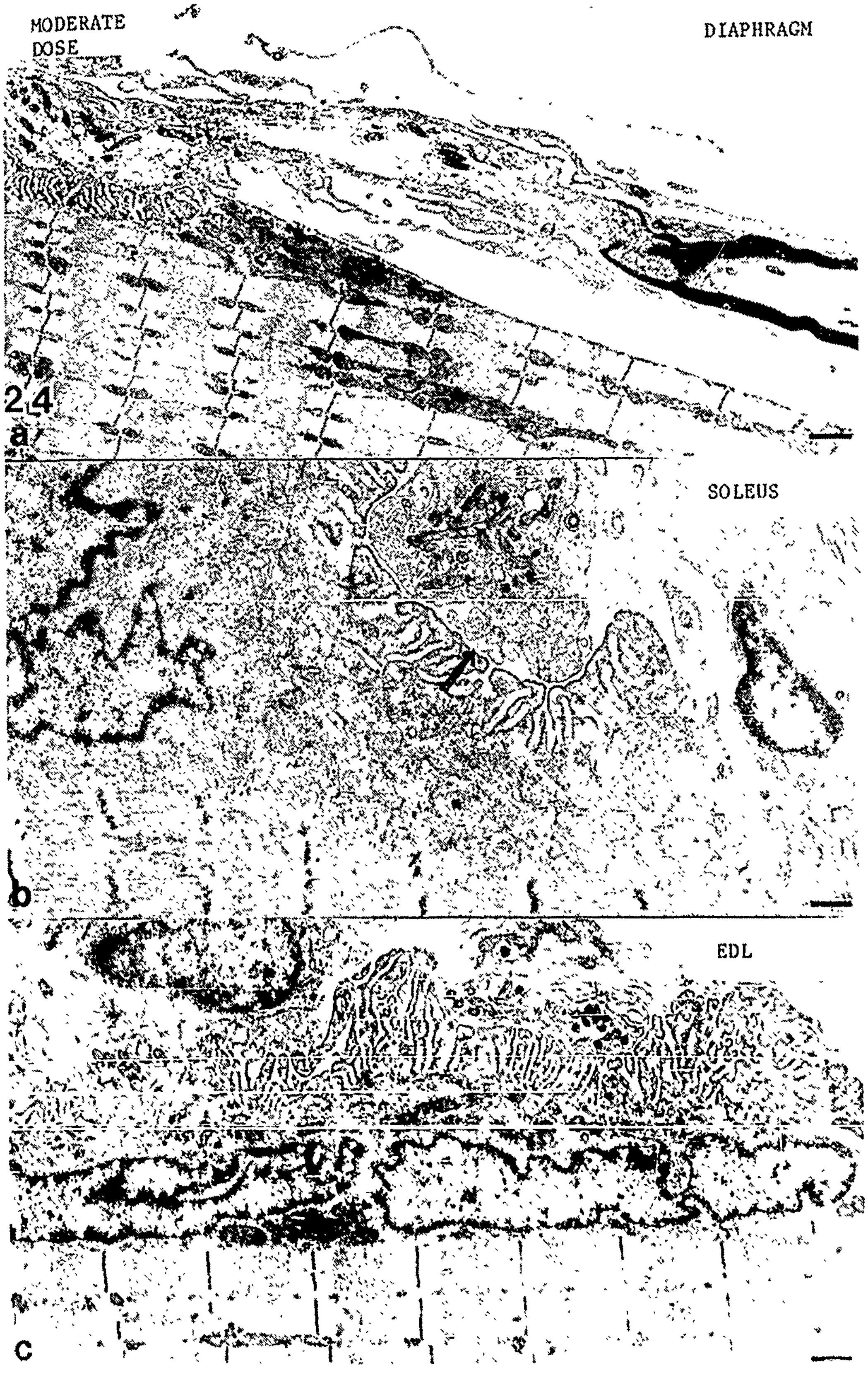


Fig. 24. Effects of an acute moderate dose of physostigmine (0.1 LD_{50}) on neuromuscular junctions 30 minutes PI. Myofibers of diaphragm (Fig. 24a) and EDL (Fig. 24c) muscles exhibit nearly normal ultrastructure, while the subjunctional sarcomeres of soleus muscle (Fig. 24b) show evidence of irregular or "zigzag" Z-bands. At this dose, most mitochondria appear normal in both subjunctional sarcoplasm and nerve terminals. Occasionally, unusual bundles of Schwann cell fingers (arrow) are interposed between the nerve terminal and junctional folds (Fig. 24b). This may represent drug effect or normal variability.

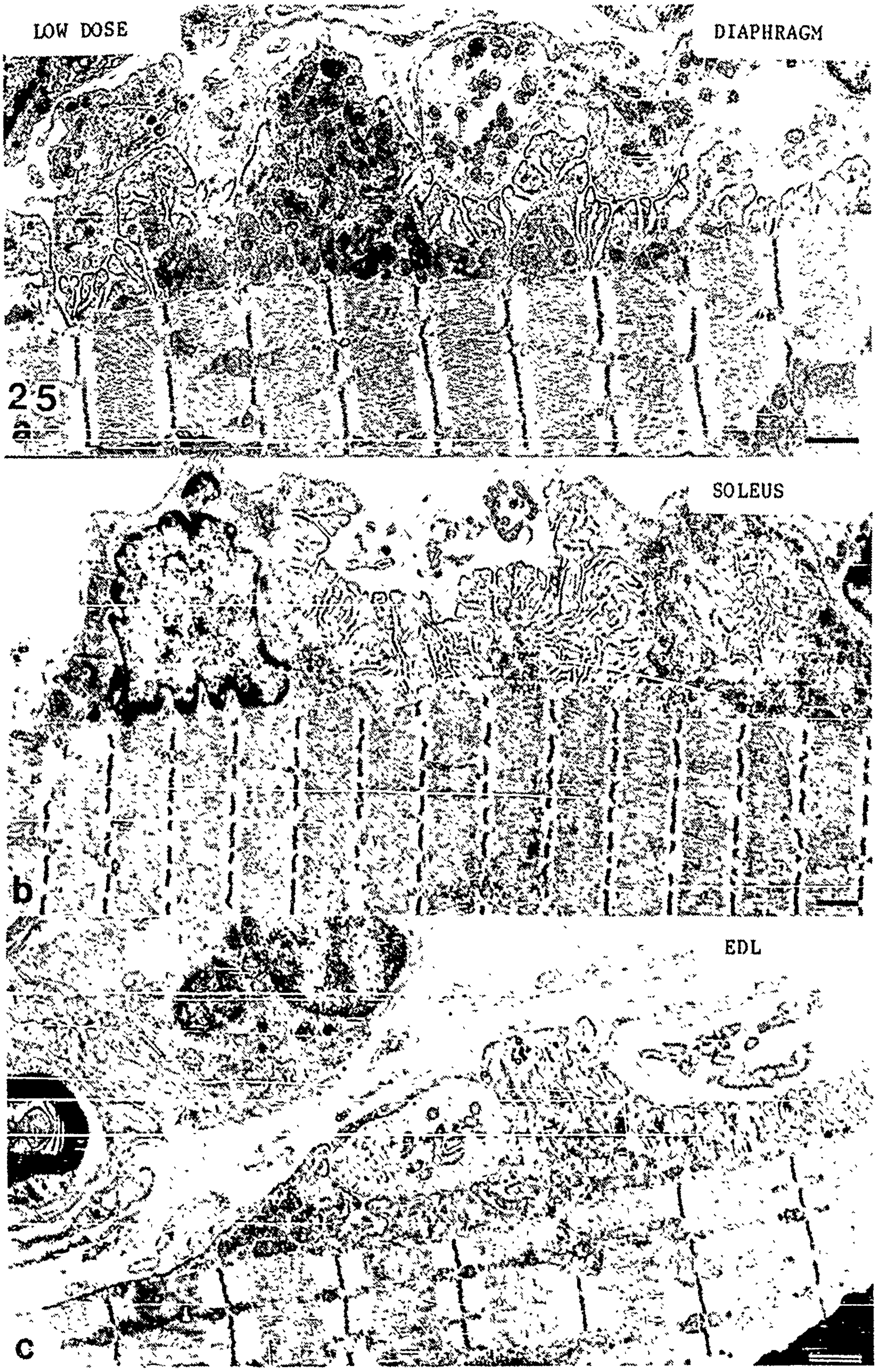


Fig. 25. Effects of an acute low dose of physostigmine (0.01 LD_{50}) on neuromuscular junctions 30 minutes PI. Myofibers and nerve terminals in the neuromuscular junctions of diaphragm (Fig. 25a), soleus (Fig. 25b, and EDL (Fig. 25c) exhibit apparently normal structure. However, a few "swollen" Mintchondria are detectable in the nerve terminals. In contrast to the high dose exposure, however, none appear "blistered" or "frothy" as would be expected to occur in the sequence leading to the type of mitochondrial swelling caused by endplate depolarization/excess Ca^{++} entry (see, for example, mitochondrial swelling due to fixation artifact. Fig. 27).

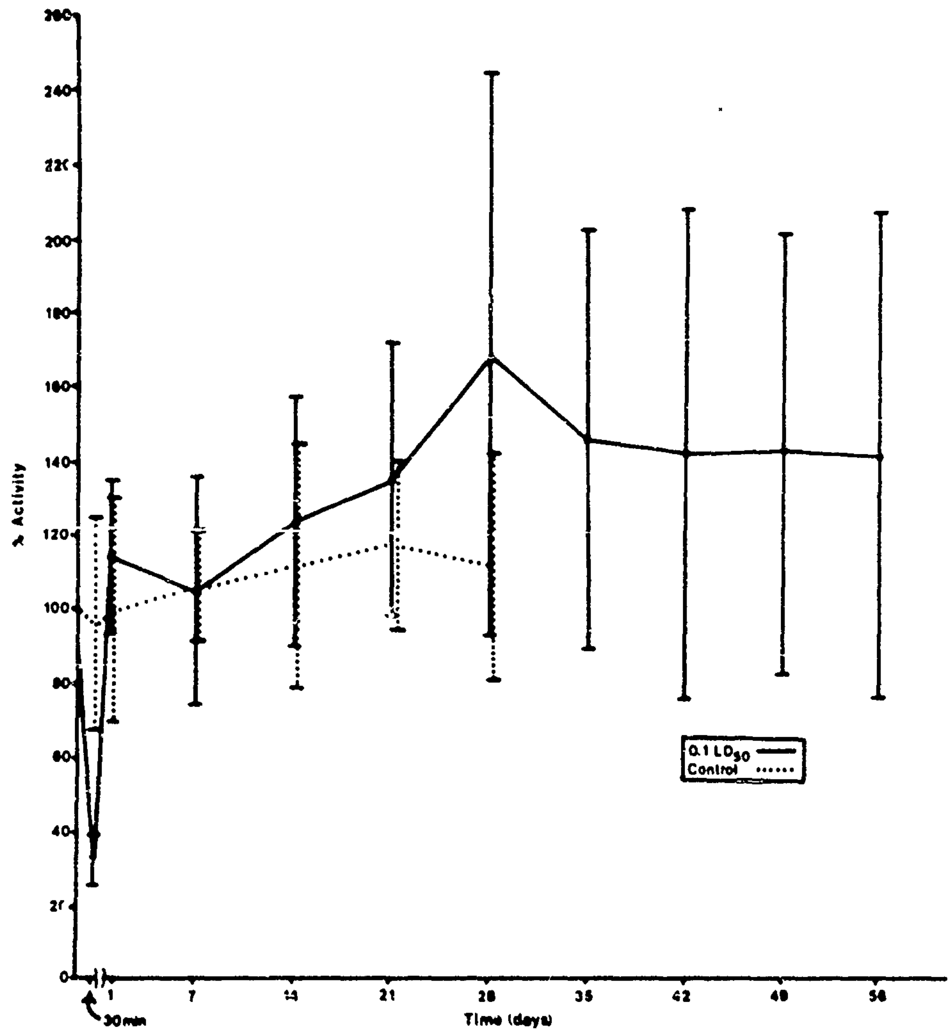


Fig. 5. Blood Cholinesterase Activity vs. Time After Injection of 0.1 LD_{50} Physostigmine.

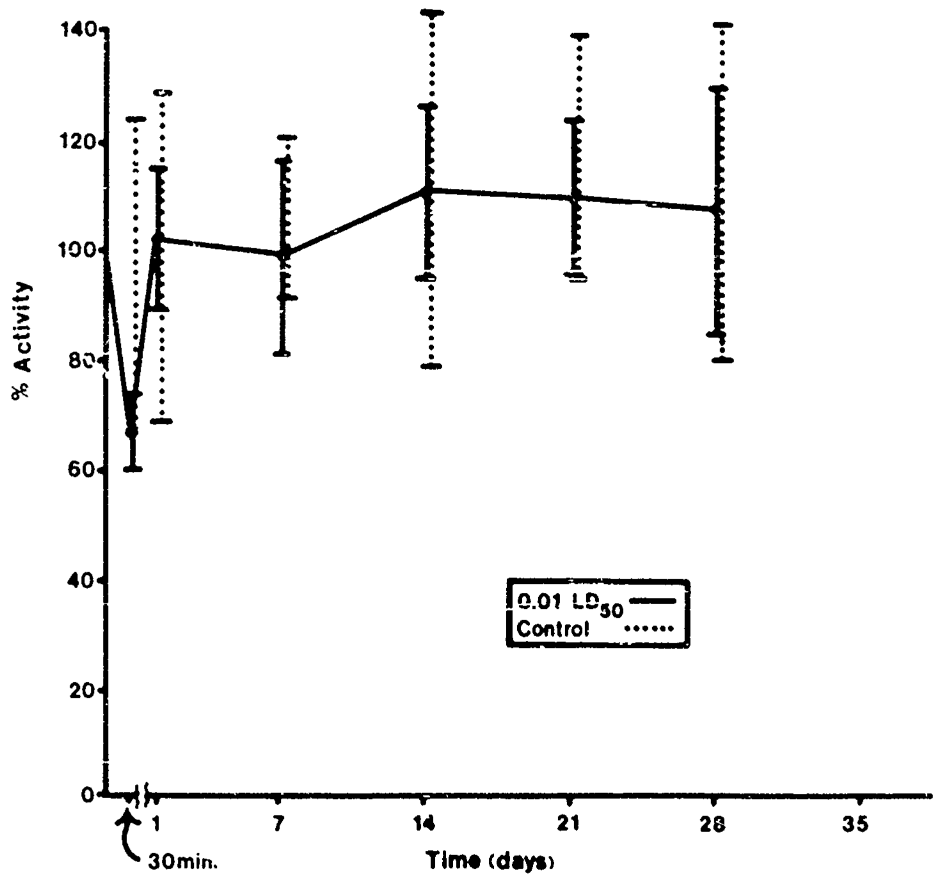


Fig. 6. Blood Cholinesterase Activity vs. Time After Injection of 0.01 LD_{50} Physostigmine.

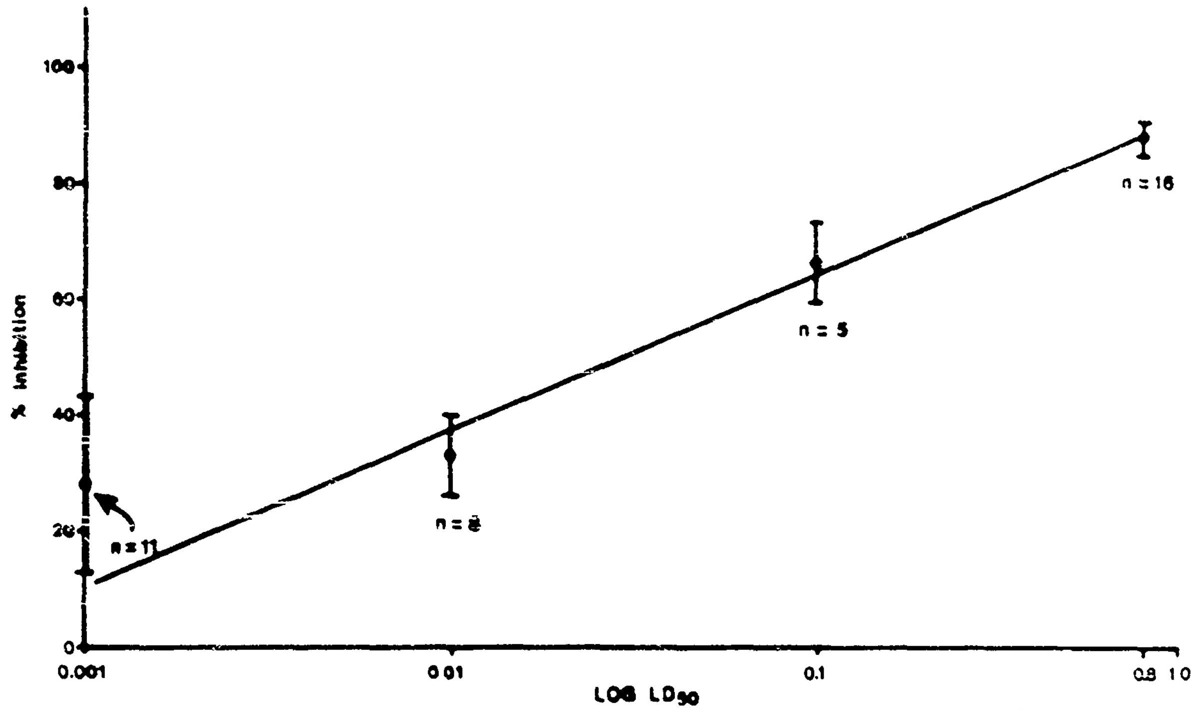


Fig. 1. Dose Response Relationship: Blood Cholinesterase Inhibition at Different Physostigmine Doses. The error bar is 1.0 standard deviation. The letter "n" refers to the number of blood samples analyzed. Each analysis was done in duplicate.

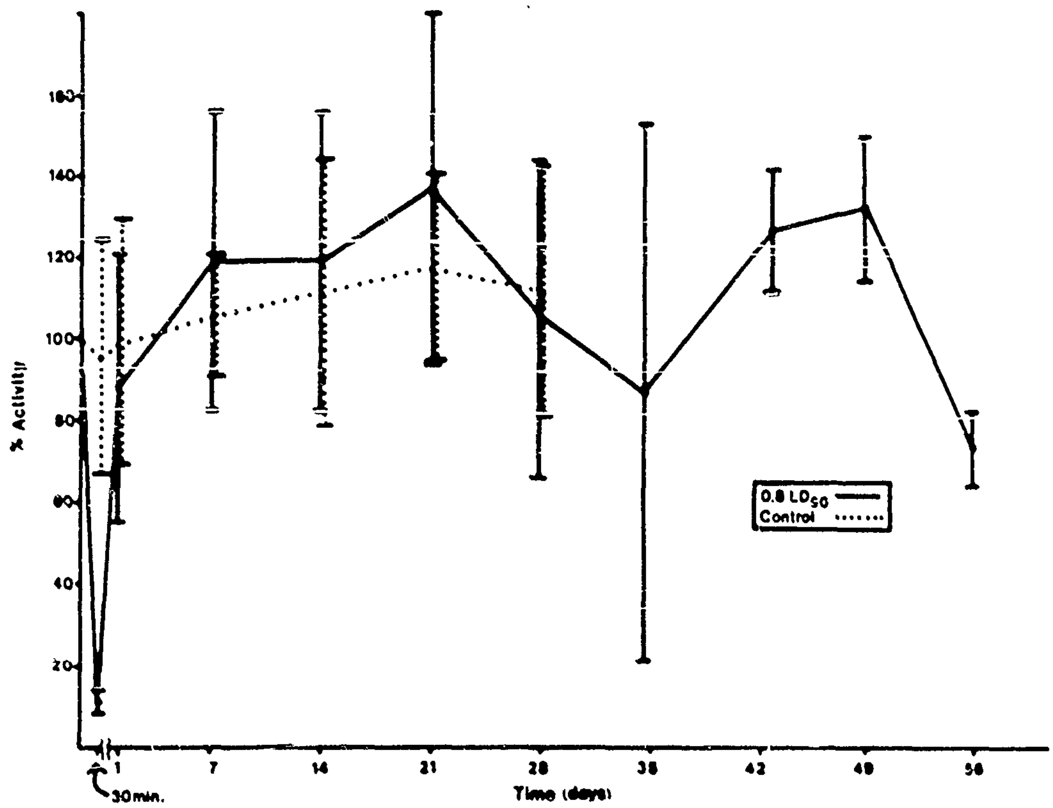


Fig. 2. Blood Cholinesterase Activity vs. Time (Post Injection of 0.8 LD_{50} Physostigmine). The error bar represents one standard deviation.

Fig. 20. Broad overview of the effects of acute high dose (0.8 LD_{50}) of physostigmine on soleus neuromuscular junction (from Fig. 18a)

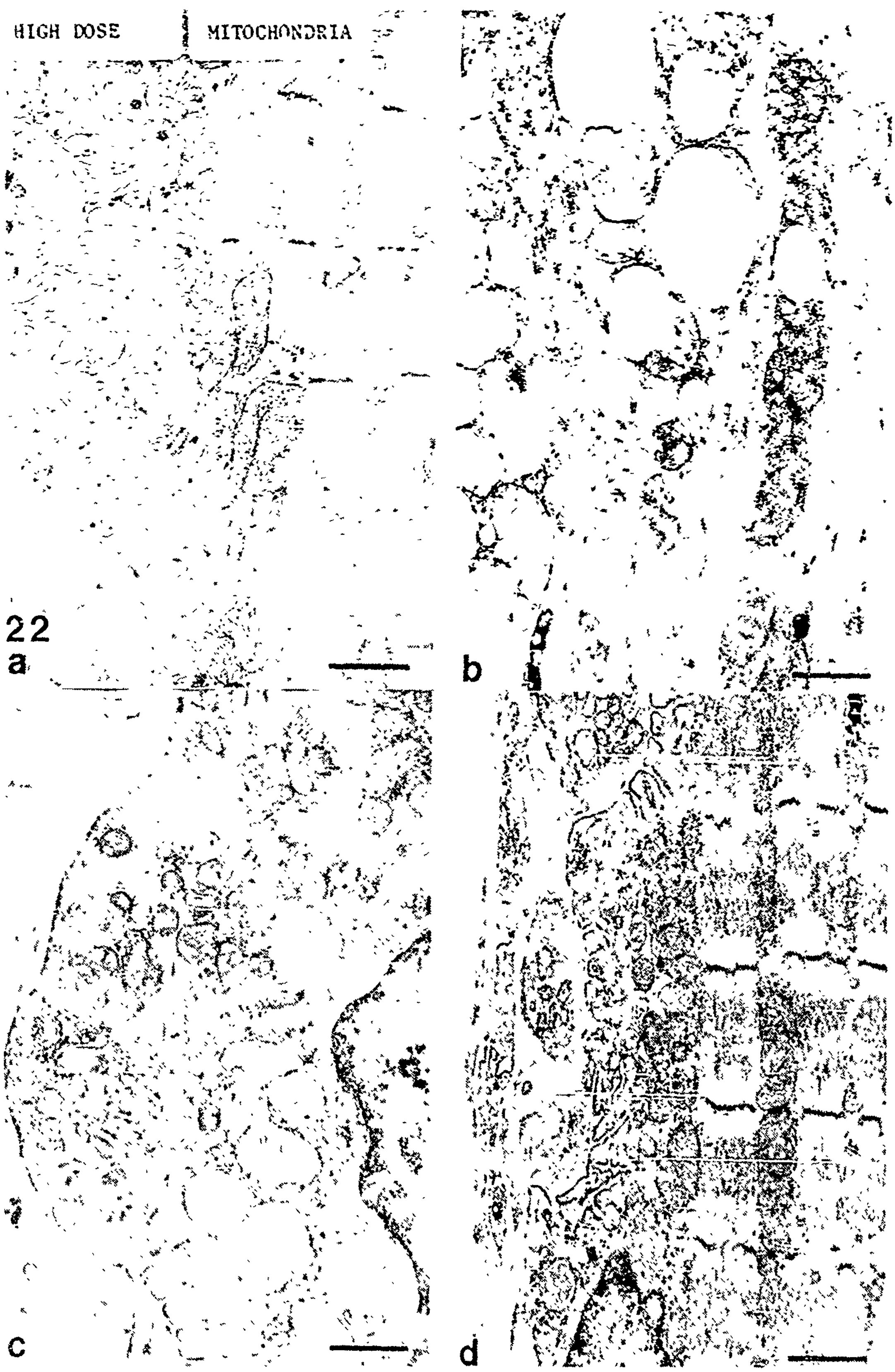


Fig. 22. Side-by-side comparison of sham injected (Fig. 22a) vs. high dose exposure (Figs. 22b-d) on endplate mitochondria. Mitochondria in sham injected animals appear normal. Acute high dose effects of physostigmine (0.8-1.1 LD_{50}) on subjunctional mitochondria 30 minutes after injection reveal numerous extremely distended mitochondria in the subjunctional sarcoplasm (Figs. 22b-d). Sarcomere damage is evident. Frothy mitochondria are also observed in the interior portion of the muscle. In soleus muscle (Fig. 22c), distended mitochondria are seen near the junctional folds, whereas "frothy" mitochondria appear at greater distances from the folds. In the EDL muscle (Fig. 22d), numerous "frothy" mitochondria and a few "blistered" or "swollen" mitochondria are seen restricted to the subjunctional sarcoplasm.

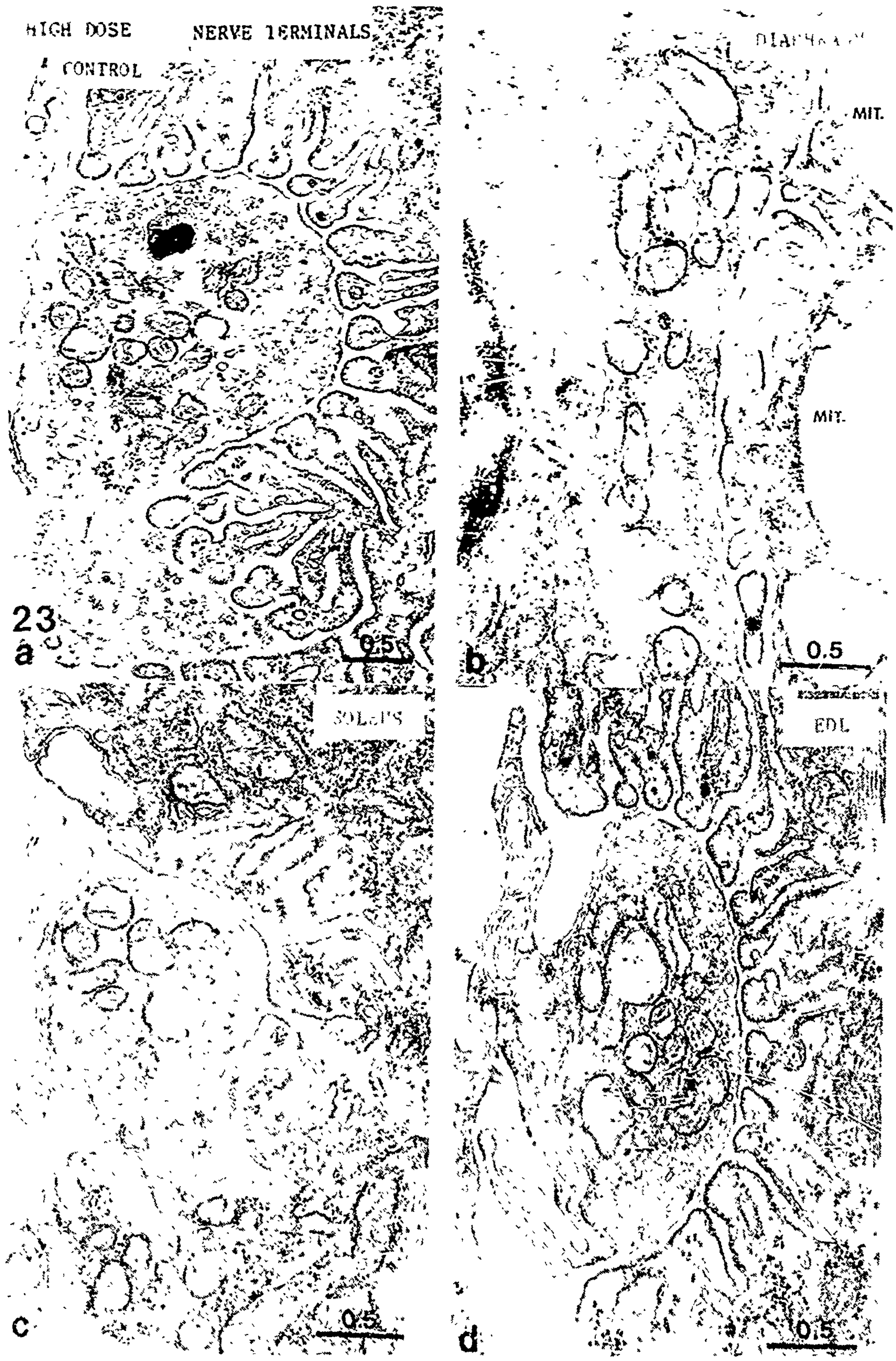


Fig. 23. Effects of an acute high dose of physostigmine (0.8-1.1 LD_{50}) on the nerve terminals of the diaphragm (Fig. 23b), soleus (Fig. 23c), and EDL (Fig. 23d) muscles 30 minutes PI. (Fig. 23 is from a "control" diaphragm.) Mitochondria are swollen within the nerve terminals of the drug-treated muscles. Swollen mitochondria are also seen in the control nerve terminal. Thus, without additional criteria, it would be difficult to determine to what extent the swollen mitochondria in the drug-treated nerve terminals is due to drug effect or to some other factor such as fixation. (But see Fig. 27 for depiction of one major criterion used to identify mitochondrial fixation artifacts.)

Table I. Number of Rats Responding to Physostigmine Administration with EDL Twitch Potentiation Under Different Anesthetics

% Showing Twitch Potentiation

Anesthesia Method	Ketamine/ Xylazine	Chloral Hydrate	Lidocaine Block
Physostigmine Dose			
0.5 LD_{50}	50% (2)*	100% (4)	100% (1)
0.25	0% (1)	67% (3)	75% (8)
0.1	0% (1)	0% (3)	100% (3)
0.05	--	--	100% (1)
0.025	--	--	0% (1)

*Numbers in parentheses are total number of animals tested.

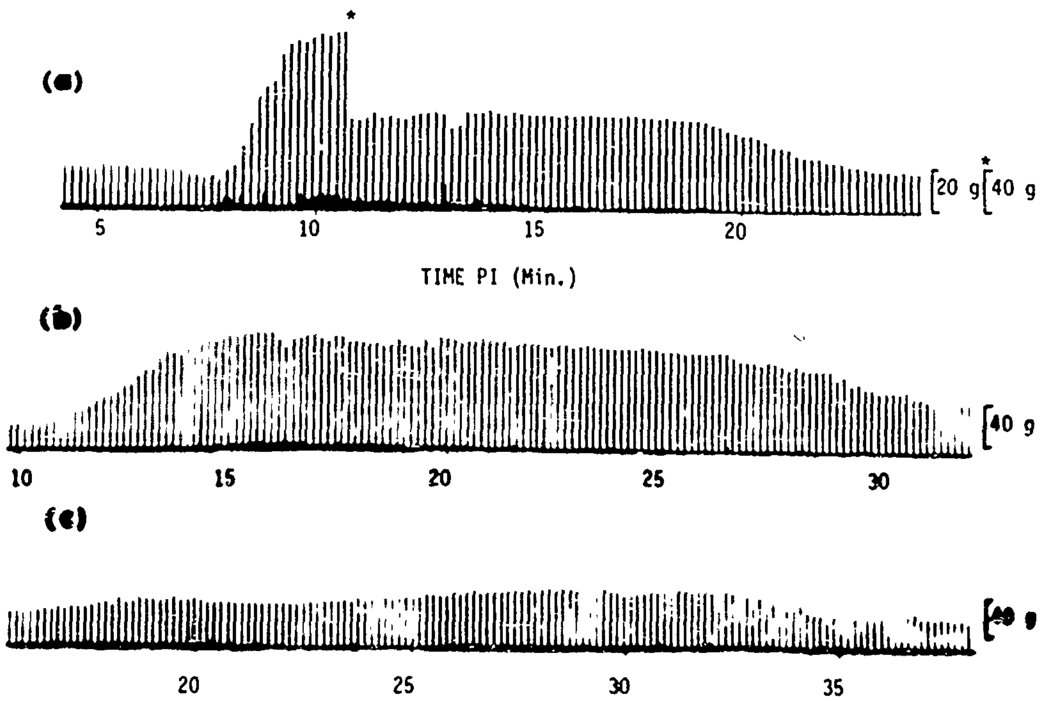


Figure 32

Fig. 32. Examples of effects of physostigmine on EDL contractility in vivo. Physostigmine was injected subcutaneously. Chart records show EDL twitch contractions in response to supramaximal stimulation of the peroneal nerve at 0.1 Hz. Twitch potentiation responses at a) 0.50 LD_{50}; amplifier gain decreased by half at * to avoid saturating recorder; b) 0.25 LD_{50}; and c) 0.05 LD_{50}. In all cases, times are minutes elapsed since physostigmine injection. All are from experiments using spinal block with lidocaine.

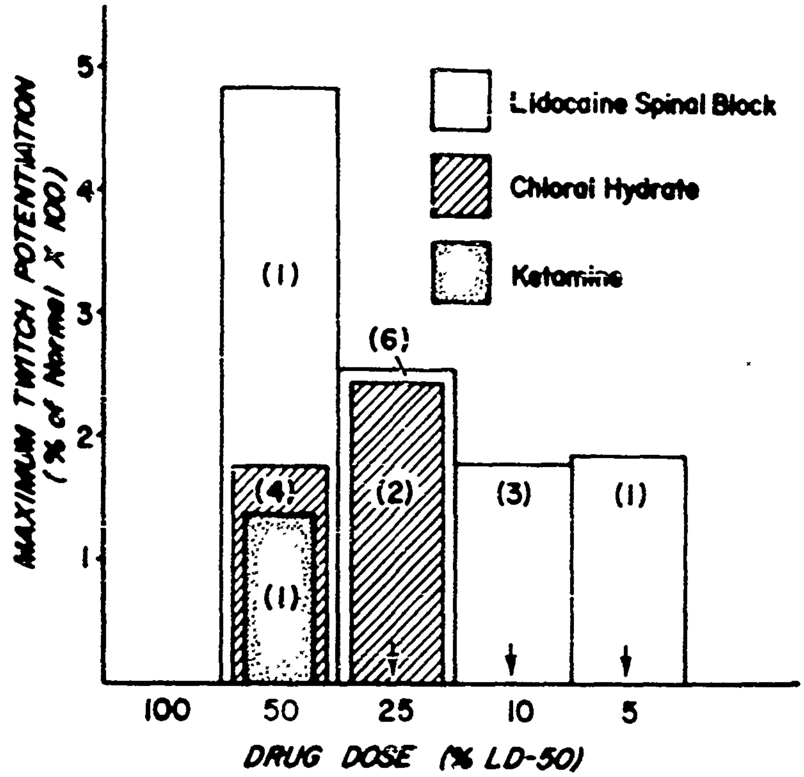


Fig. 33. Maximum in vivo EDL twitch potentiation in response to acute physostigmine administration under different anesthetics. Physostigmine was injected subcutaneously. Numbers in parentheses indicate total number of rats/EDL muscles showing a potentiation response. Downward arrows indicate that there were no positive responses for ketamine anesthesia at 0.25 LD_{50} or for ketamine or chloral hydrate at 0.1 LD_{50} and 0.05 LD_{50}.

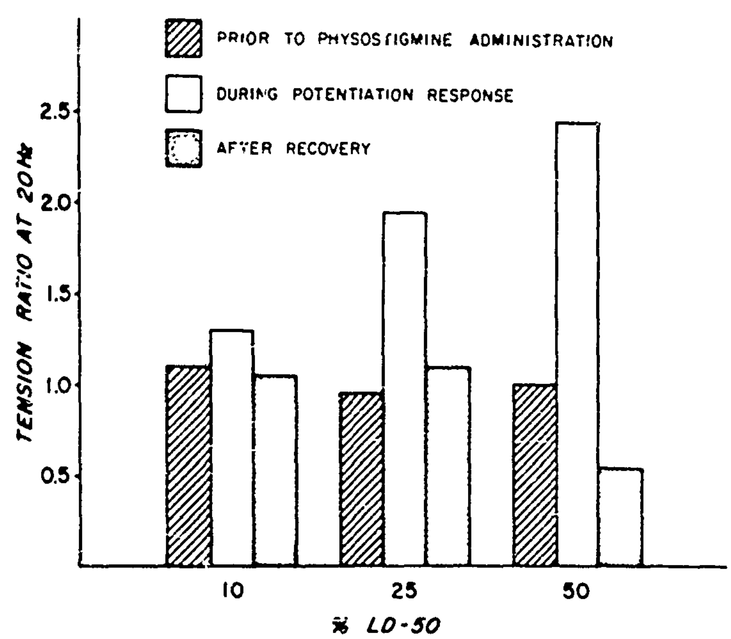


Fig. 34. Effect of physostigmine on ability of EDL to sustain high frequency contraction. Physostigmine injected subcutaneously. The "tension ratio" is the tension (in grams) at the beginning of a 10 second 20 Hz stimulus train divided by the tension at the end of the train. Higher "tension ratios" indicate decreased ability to sustain contraction.

Fig. 26. Effects of an acute low dose of physostigmine (0.001 LD_{50}) on neuromuscular junctions 30 minutes PI. Myofibers of diaphragm (Fig. 26a), soleus (Fig. 26b), and EDL (Fig. 26c) appear normal. In the soleus muscle, mitochondria appear normal in both nerve terminals and subjunctional sarcoplasms. However, many distended mitochondria were seen in the nerve terminals of diaphragm and EDL muscles. Based on high magnification analysis of the same endplates (see Fig. 27), we attribute this type of swollen mitochondria to artifact of hypoxia/glutaraldehyde fixation (see Figs. 11 and 27).

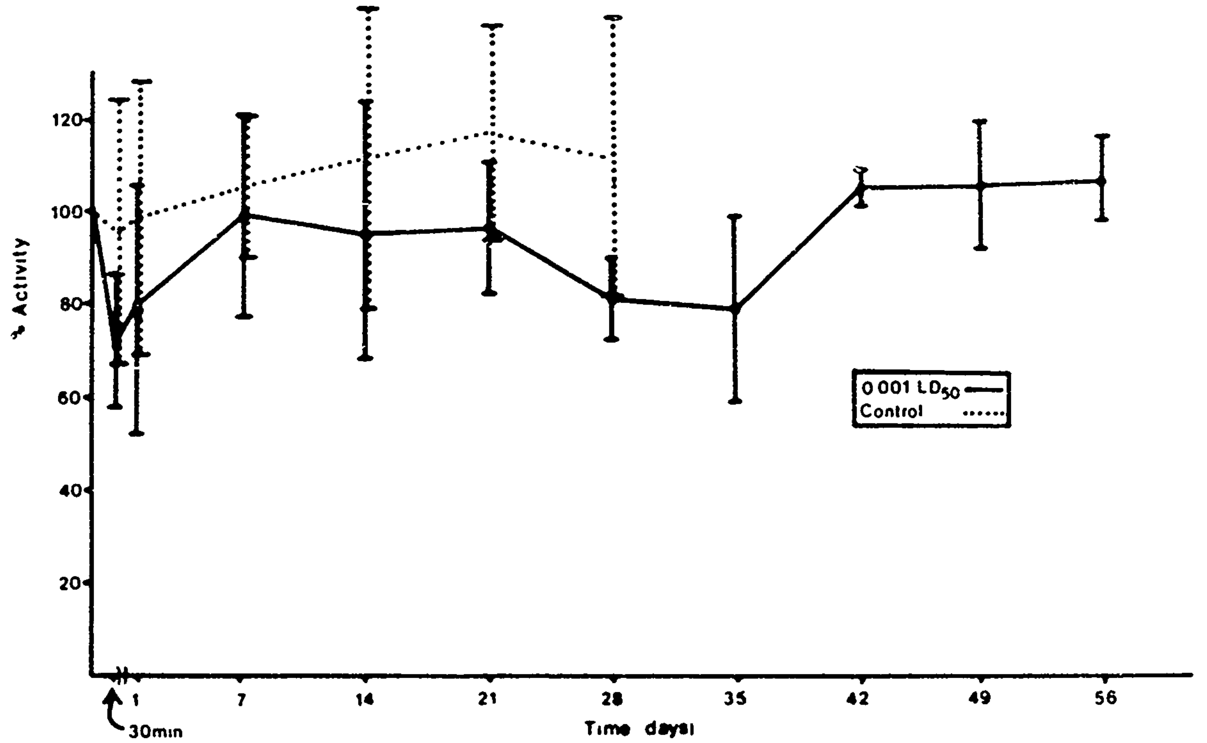


Fig. 7. Blood Cholinesterase Activity Vs. Time After Injection of 0.001 LD_{50} Physostigmine.

Fig. 28. Effects of an acute high dose of physostigmine (0.8-1.1 LD_{50}) on the neuromuscular junctions 1 day PI. In the diaphragm (Fig. 28a), an irregular arrangement of myofibrils is seen just below the endplate. Junctional folds appear slightly broadened. The myofibrils appear almost fully recovered in this area. There are no swollen or frothy mitochondria in evidence. In the soleus muscle (Fig. 28b), the neuromuscular junctions and myofibrils appeared normal. Since 100% of diaphragm and soleus myofibers had been affected at 30 minutes (Figs. 13 and 14), it is clear that the muscle fibers are capable of extremely rapid recovery following a single high dose injection. However, in the EDL muscle (Fig. 28c) the junctional folds appear abnormally broadened, and extensive areas of damaged myofibrils having very irregular Z bands and frothy mitochondria were observed. We infer that additional damage occurred in EDL fibers between 30 minutes and approximately 6 hours (the time to inactivate physostigmine), but that much of the damage had been repaired by 1 day PI.

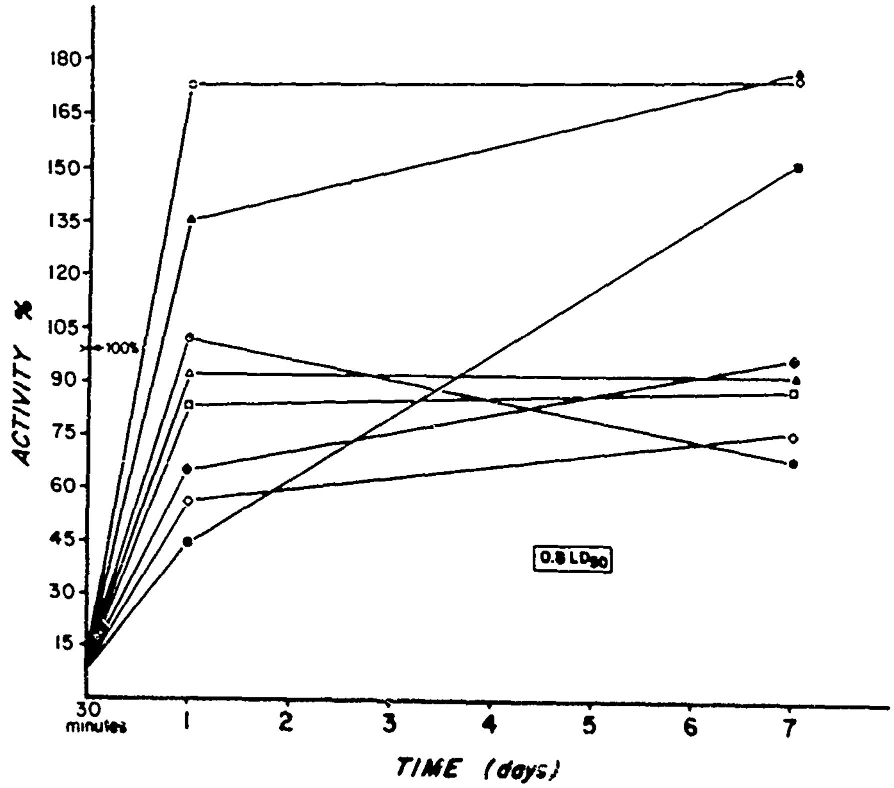


Fig. 3. Blood Cholinesterase Activity vs. Time After Injection of 0.8 LD_{50} Physotigmine. Enzyme profiles for individual rats showing individual variation over a 1 week period PI.

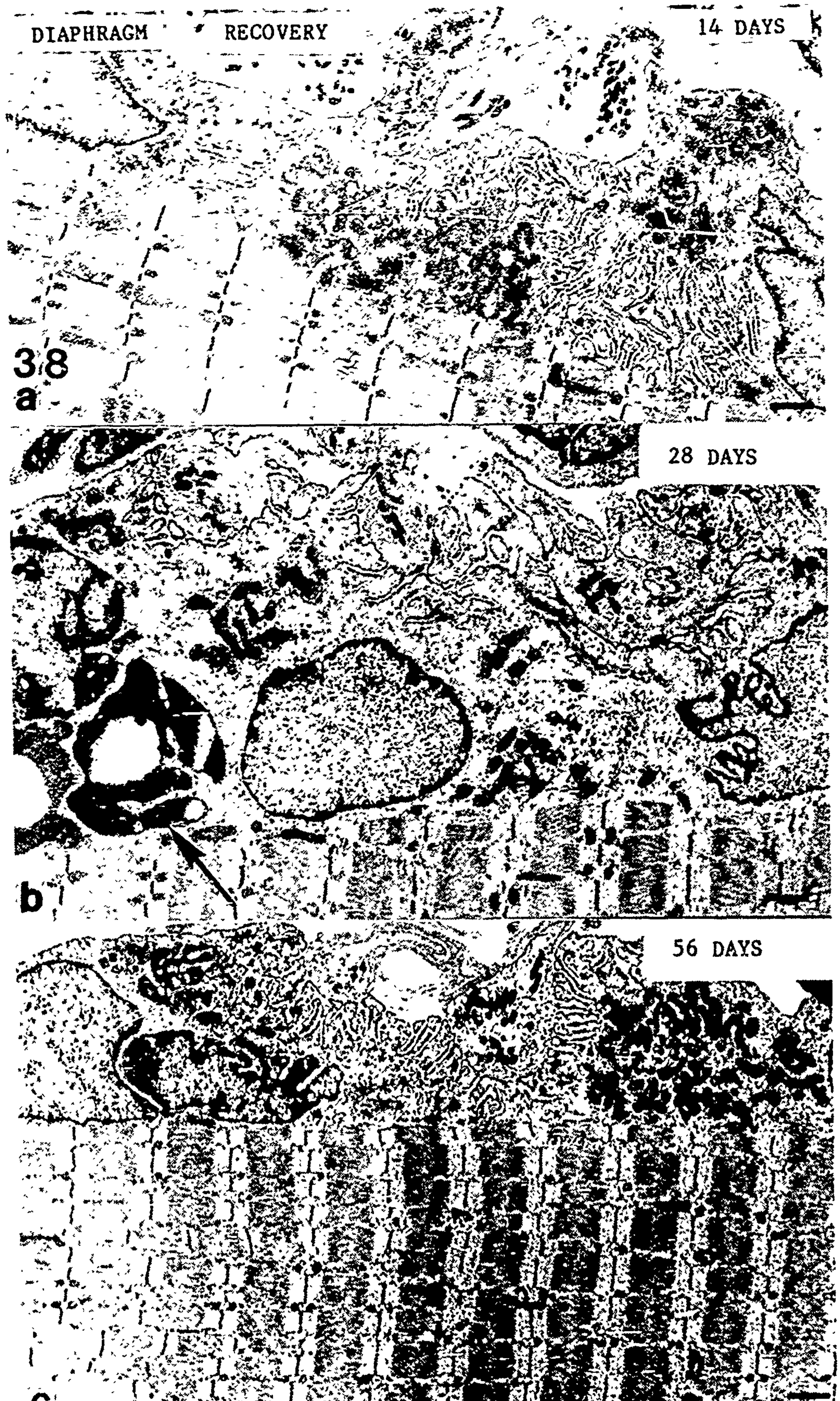


Fig. 38. Recovery of diaphragm neuromuscular junctions 14 days (Fig. 36a), 28 days (Fig. 36b), and 56 days (Fig. 36c) after acute high-dose exposure to physostigmine (0.8-1.1 LD_{50}). Endplate morphology appears normal. The "arrow" in Fig. 36b points to the necrotic residue of a nucleus. No other structural alterations were detected in these muscles.

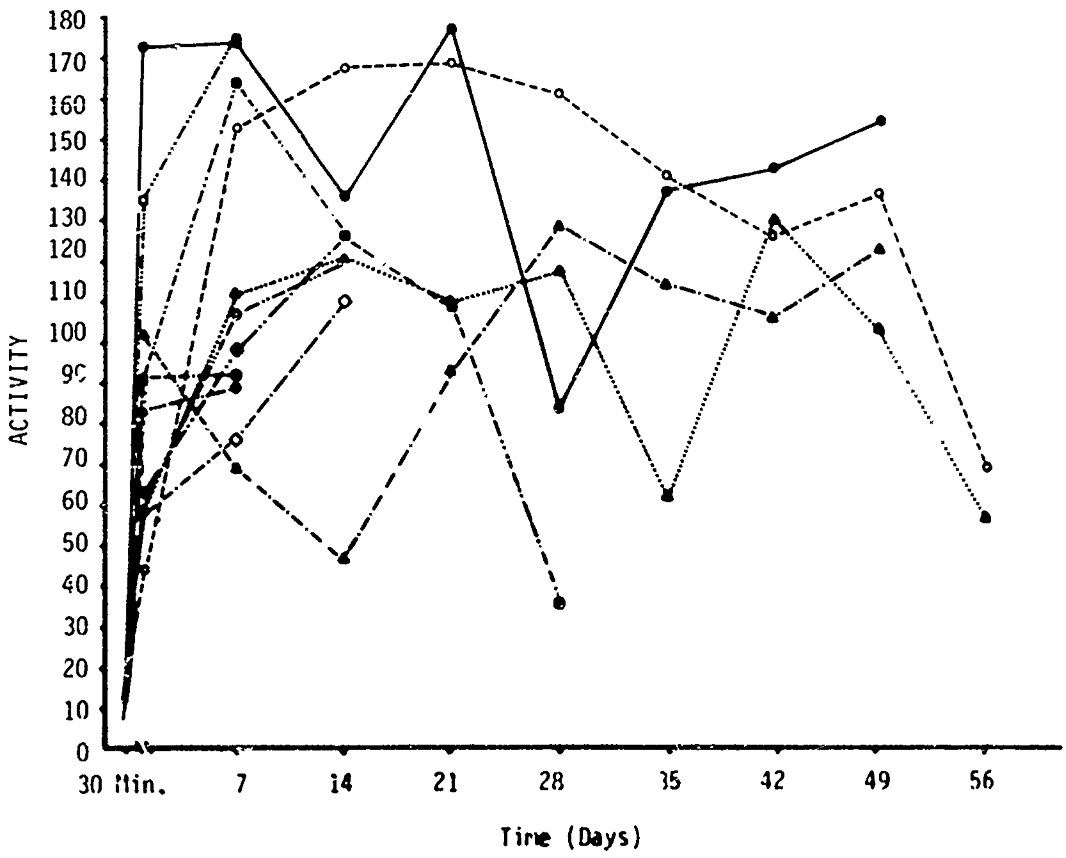


Fig. 4. Blood Cholinesterase Activity vs. Time After Injection of 0.8 LD_{50} Physotigmine. Enzyme profiles for some individual rats showing individual variation up to 8 weeks PI.

Fig. 10. Identification of artifacts of fixation. Poor perfusions are characterized by the presence of numerous erythrocytes in many capillaries (Fig. 10a) and by the presence of characteristic "swollen" or "exploded" mitochondria in all areas of the myofibers, in the nerve terminals, and in their myelinated axons (Fig. 10b, arrowheads). These data are useful in distinguishing between structural alterations caused by physostigmine vs. those resulting from fixation artifacts.

Fig. 27. Higher magnification image of the endplate depicted in Fig. 26a. Whether in nerve terminals (this figure), skeletal muscle (Fig. 11 and Fig. 26c), or cardiac muscle (Rash et al, 1974), hypoxia during fixation has been shown to result in severe mitochondrial swelling and engulfment of glycogen granules. When tissues are stained en bloc with aqueous uranyl acetate (Fig. 1 from Rash, 1974), the glycogen granules are dissolved, leaving characteristic strings of tiny electron dense granules within the distended mitochondrial matrix (see Fig. 27, asterisk). Whether the tissue hypoxia resulted from hyperactivity, reduced circulation, or secondary drug effect has not yet been determined. However, the endplates from rats treated with low dose and very low dose of physostigmine seemed especially susceptible to this "artifact."

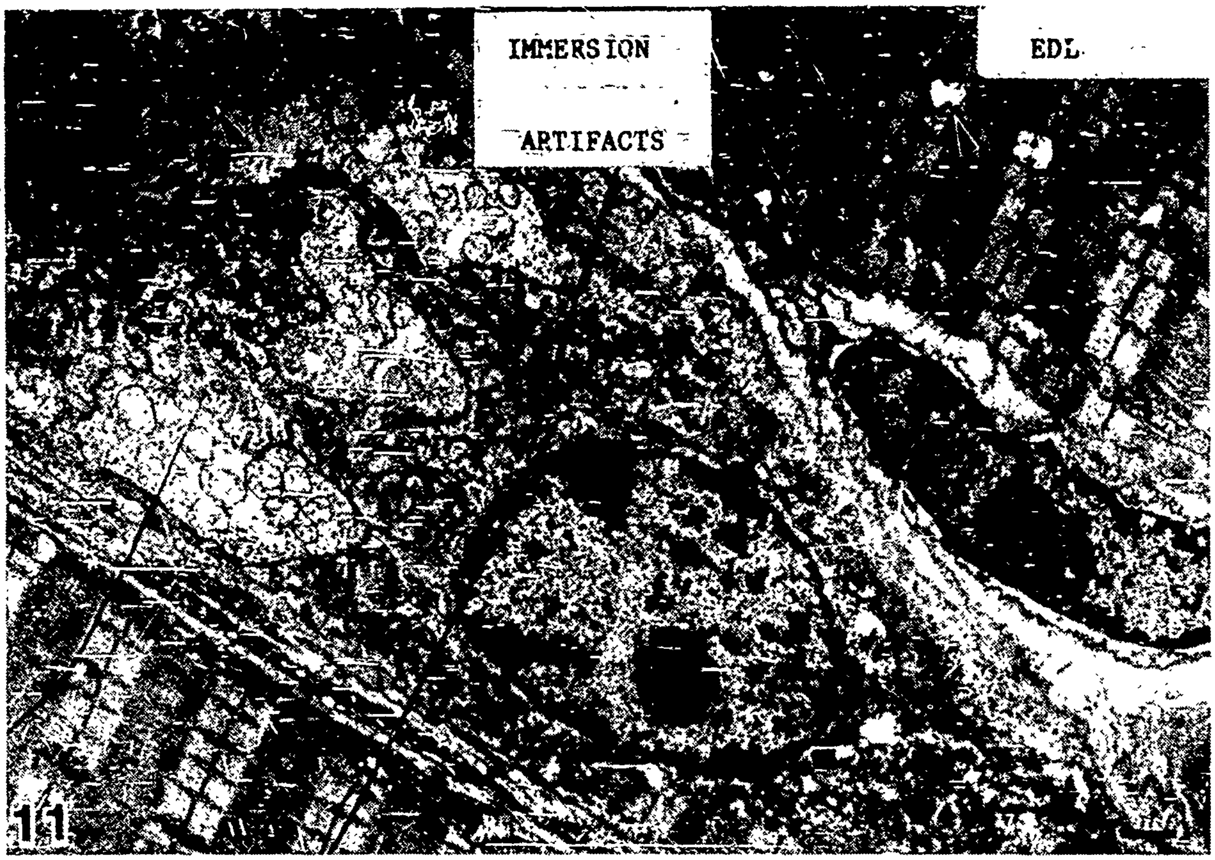


Fig. 11. Immersion fixation used to identify artifacts of fixation. In immersion-fixed "control" muscles, many "swollen" or "exploded" mitochondria are seen in all areas of the myofibers and in the nerve terminals (arrowheads). Some swollen cisternae of sarcoplasmic reticulum are also seen. These artifacts of fixation must be recognized and clearly distinguished from effects of physostigmine.

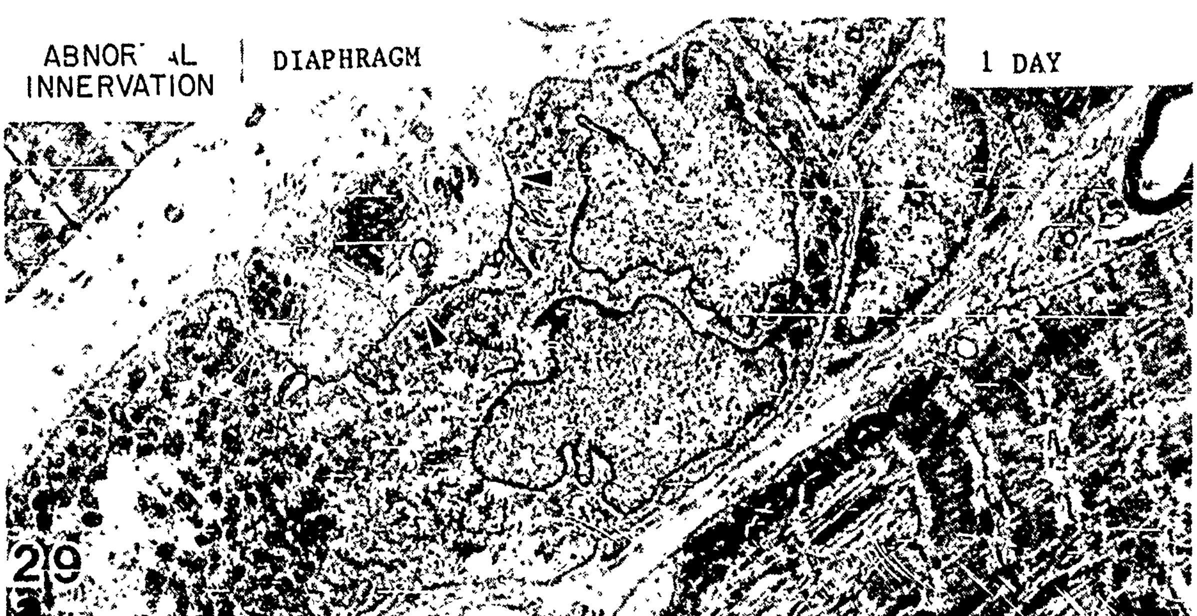
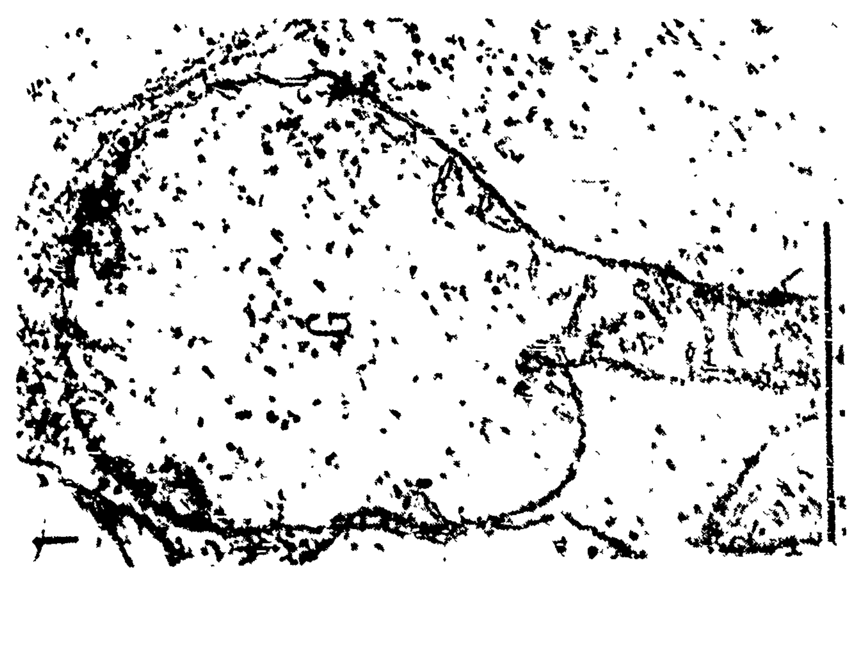


Fig. 29. Recovery of diaphragm neuromuscular junction 1 and 7 days after high dose physostigmine (0.8-1.1 LD_{50}). The mitochondria appeared normal, but continuing evidence of myofibril destruction both at the endplate and away from the neuromuscular junction was observed. Unusual clusters of nerve terminals (arrowheads) were also seen at 1 day PI.



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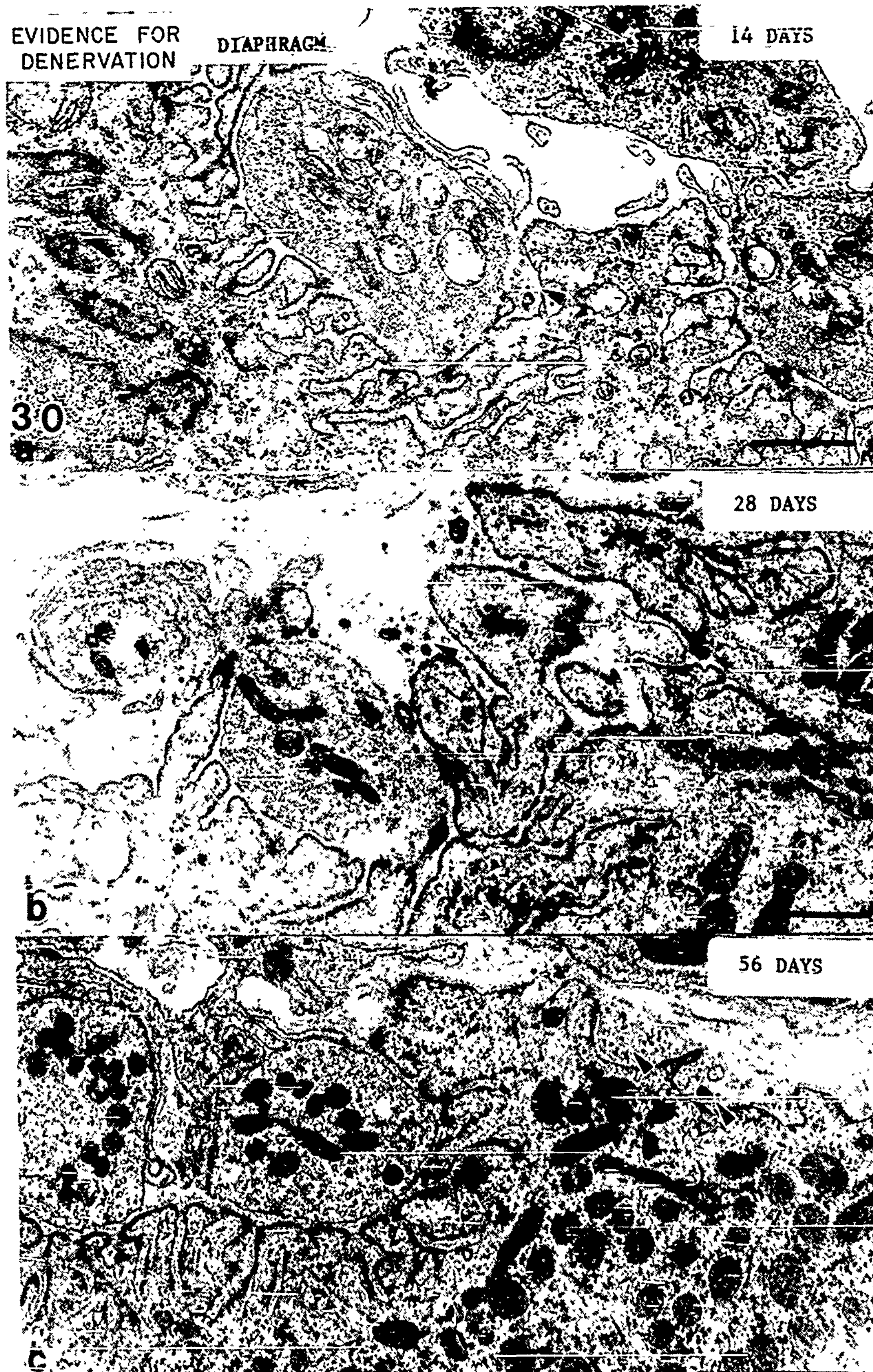

Fig. 30. Delayed effects on the nerve terminals of diaphragm muscle after a single acute high dose injection of physostigmine. Micrographs show neuromuscular junctions of diaphragm muscles 14 days (Fig. 30a), 28 days (Fig. 30b), and 56 days (Fig. 30c) PI. Debris (arrowheads) similar to that reported in a neuromuscular junction from human patients with myasthenia gravis and all rats treated with anti-ChE agents (see text) are found in the synaptic cleft (Fig. 30a), on the degnerative surface of the junction (Fig. 30b), and on the denervated surface of a degenerated endplate (Fig. 30c).

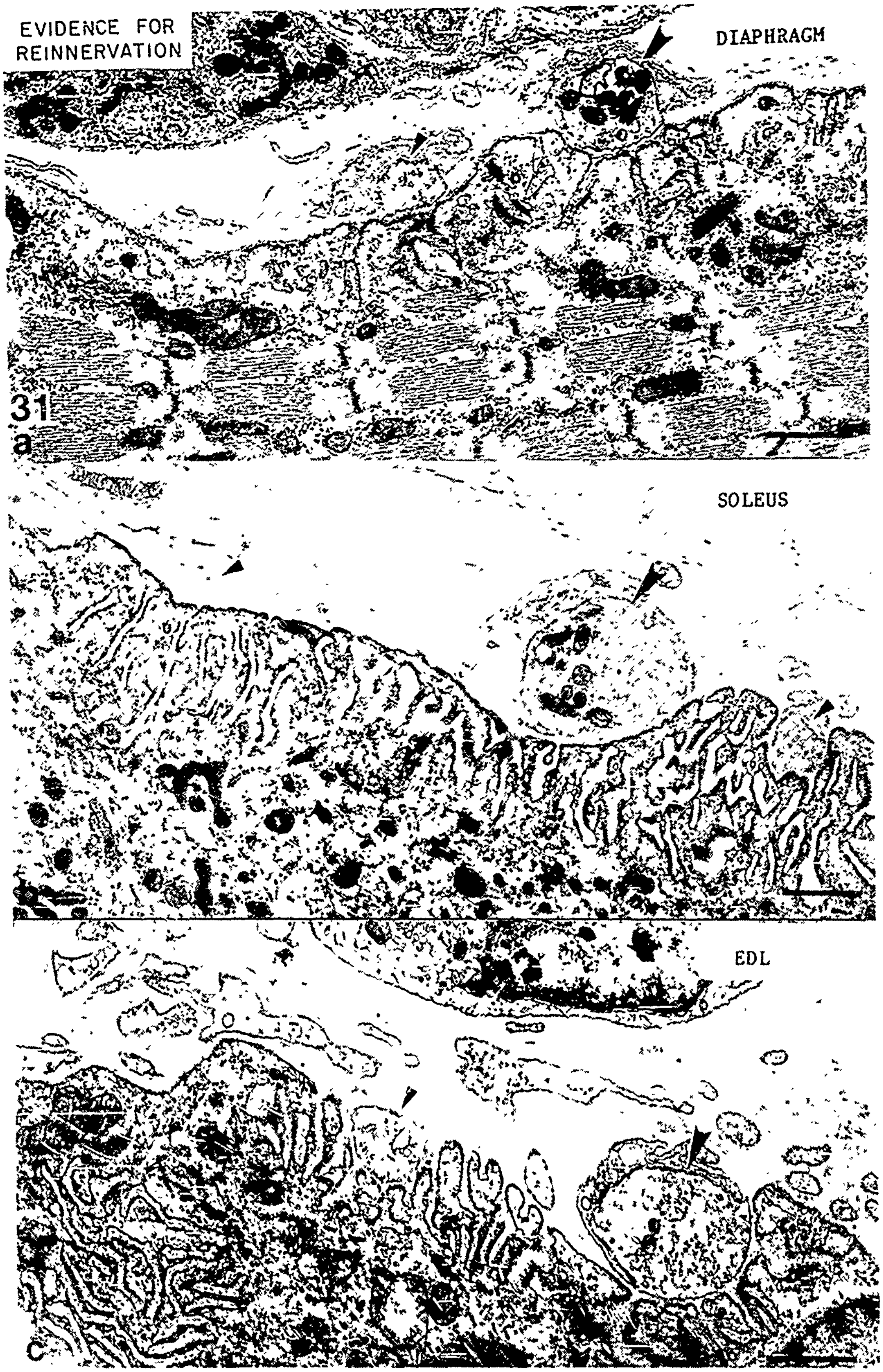

Fig. 31. Delayed effects of acute high dose exposure to physostigmine on the nerve terminals of diaphragm (Fig. 31a) and soleus (Fig. 31b) muscles 1 day PI and of EDL muscle (Fig. 31c) 7 days PI. Small nerve terminals (small arrowheads) and larger nerve terminals (large arrowheads) were observed on and adjacent to the flattened denervated portions of the junction. These micrographs are consistent with processes of denervation and reinnervation of nerve terminals by collateral "sprouting."

EFFECTS OF ANTICHOLINESTERASE AGENTS ON HIPPOCAMPAL SLICES FROM THE RAT

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INTRODUCTION

It has been known for several years that the carbamate anticholinesterases, such as eserine, and the organophosphate anticholinesterases, such diisopropylfluorophosphate (DFP) and soman, can produce seizure activity in the central nervous system (CNS). While some of this activity is probably the result of acetylcholine (ACh) accumulation subsequent to acetylcholinesterase inhibition, it is possible that DFP and soman also produce excitatory effects by other mechanisms. In fact, increasing the concentration of organophosphate above that required to completely inhibit acetylcholinesterase produces increasing CNS excitation leading to seizures (Karczmar, Fund. Appl. Toxicol. 4:S1, 1984).

We have chosen to examine the excitatory effects of eserine, DFP, and soman, using the rat hippocampal slice as a model. This slice possesses several advantages. First, the hippocampus is prone to seizure activity elicited by a number of mechanisms. Second, the hippocampus is known to possess cholinergic innervation in several fields. Third, ACh is known to facilitate the action of other excitatory inputs (Krnjevic & Ropert, Neurosci. 2:2165, 1982). Fourth, most of the excitatory effects of ACh in the hippocampus are blocked by a single pharmacological agent, the muscarinic antagonist, atropine. Therefore, it should be possible to use atropine to block the effect of ACh accumulation, thereby unmasking excitatory effects due to other mechanisms.

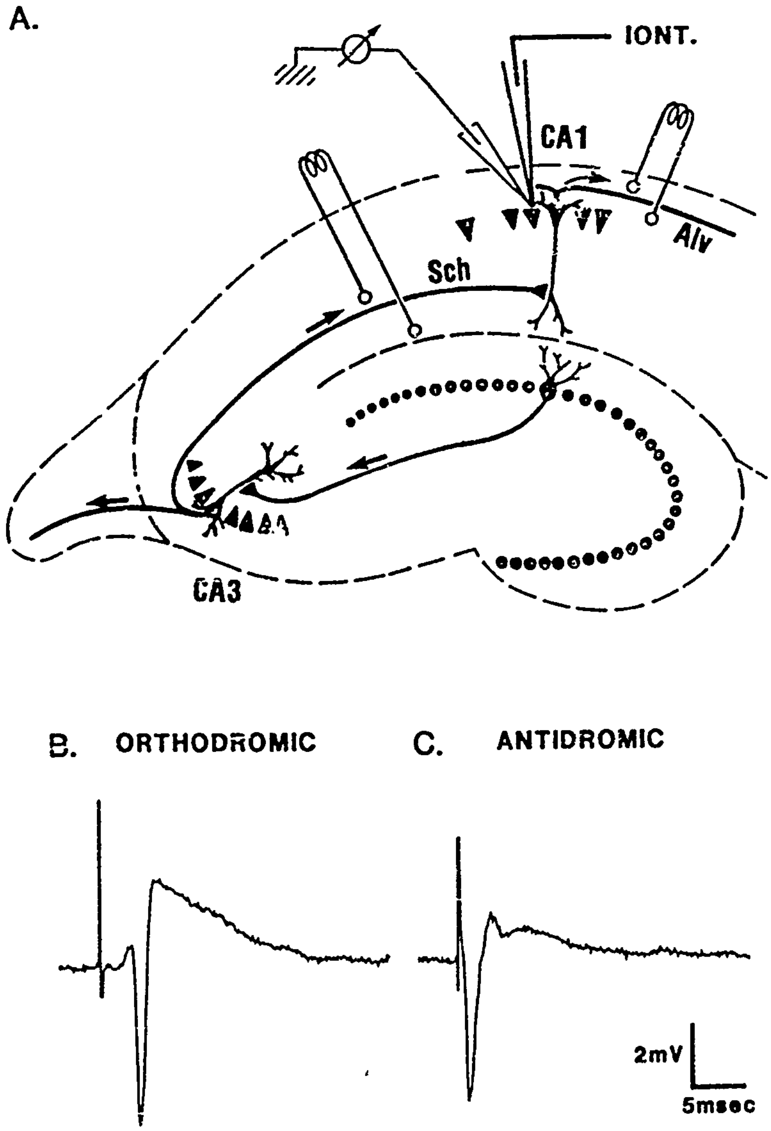
METHODS

Hippocampal slices (400 um thick) were prepared from male Sprague-Dawley rats (150-250 g.). The slices were incubated for at least 30 minutes at 30° C in Krebs- Ringer buffer with the following composition (mM): NaCl, 124; KCl, 5.0; $CaCl_2$, 2.4; KH_2PO_4, 1.3; $MgSO_4$, 1.3; $NaHCO_3$, 26; and glucose, 10. The solution was continuously bubbled with 95 % O_2 / 5 % CO_2. Experiments were conducted in a submerged slice chamber designed by Dr. Kerry Zbicz, NIAAA. The flow rate of fresh buffer through the chamber was 1-3 ml/min. Temperature was maintained between 30 -32° C.

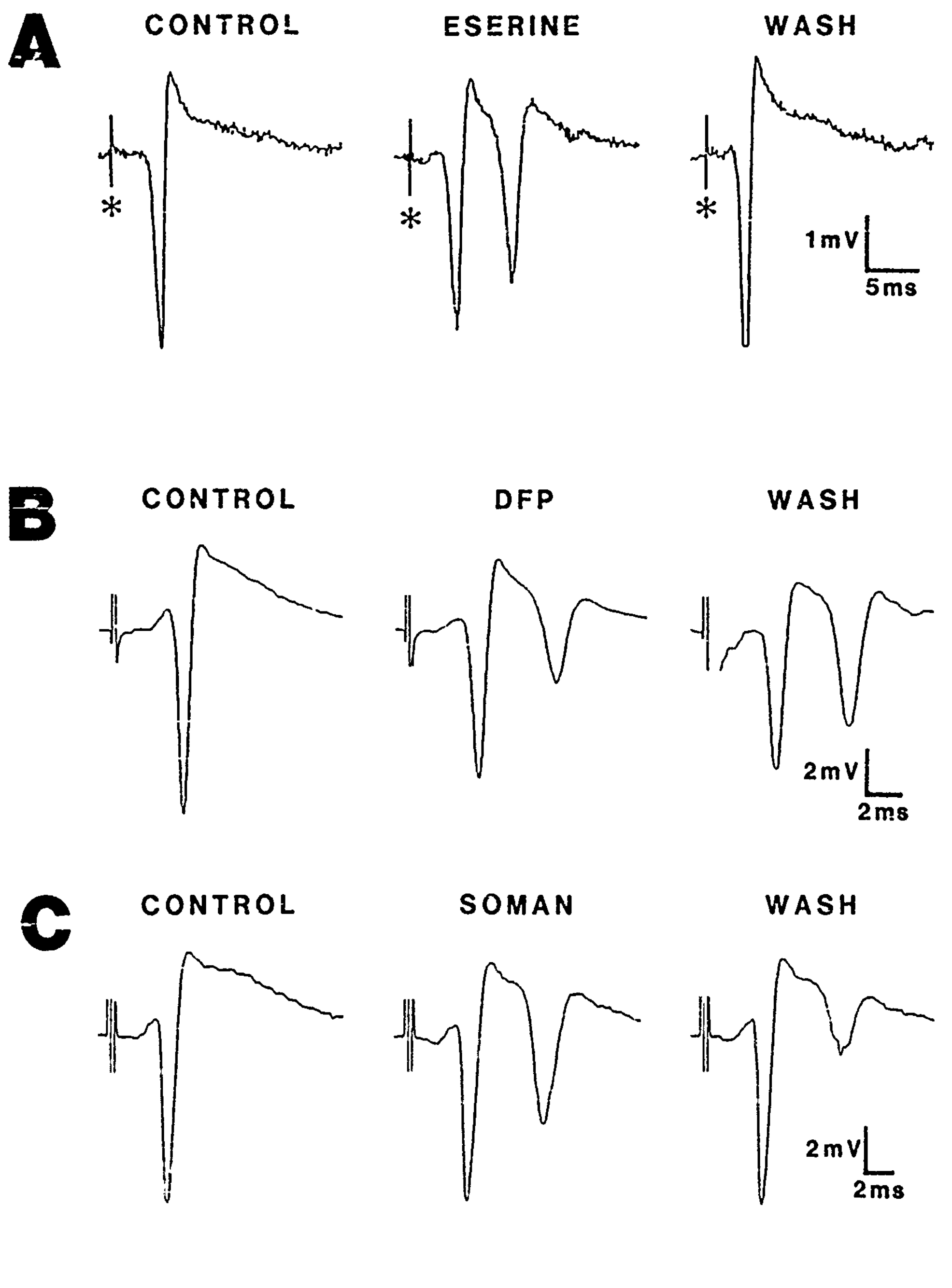
Extracellular population spikes (the summed action potentials of the cells surrounding the recording electrode) were recorded with glass micropipettes in the cell body layer of field CA1. Figure 1a shows the hippocampal slice with the usual placement of recording and stimulating electrodes. Orthodromic responses (fig 1b) were obtained by stimulation of the Schaffer collateral fibers (Sch) in the stratum radiatum. Antidromic responses (fig. 1c) were elicited by stimulation of the alveus (Alv).

In several experiments, ACh was applied in the cell body layer by iontophoresis using a constant current unit (Medical Systems Corp.). The placement of the iontophoretic electrode is shown in fig. 1a. Responses to iontophoretically applied ACh were considered stable if they were reproducible at 5 minute intervals over a 20 minute control period before application of atropine. To control for current artifacts, the polarization of the ejecting current was reversed, and a pulse was applied. That procedure produced no effect on the amplitude or shape of the population spike.

Amplitudes of the orthodromic population spikes were measured by the method of Alger and Teyler (Brain Res. <u>110</u>: 463, 1976). Antidromic population spikes were computed as the voltage change from baseline to the maximum negativity immediately following the stimulus artifact. Statistical analyses were performed using two-tailed paired T-tests, and differences were considered significant at $p < .05$.


A.
IONT.
CA1
Alv
Sch
CA3
B. ORTHODROMIC
C. ANTIDROMIC
2mV
5msec

EFFECTS OF ESERINE, DFP, AND SOMAN



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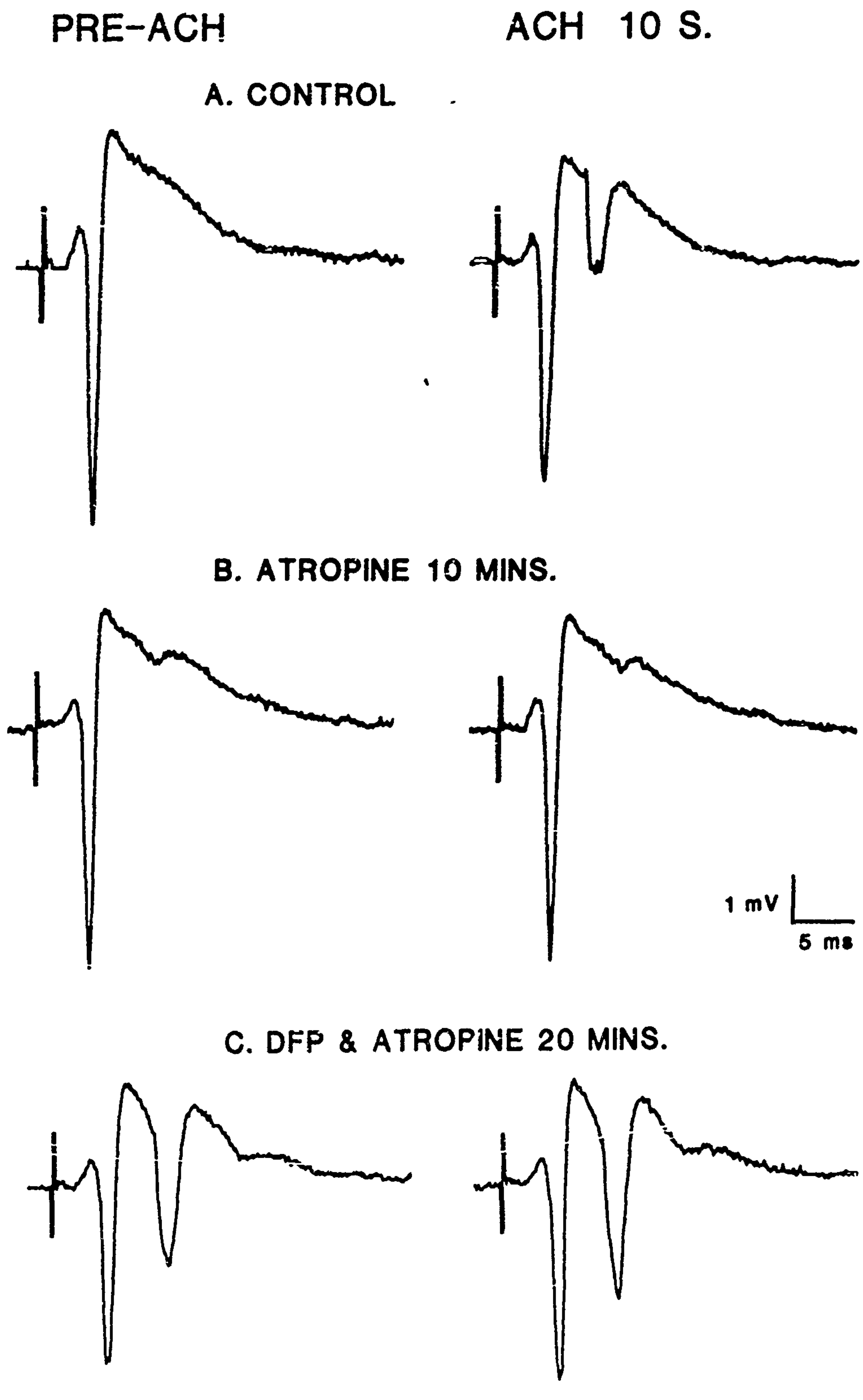
A. **The carbamate anticholinesterase, eserine (10 µM), applied for 20 min, elicited a large second population spike, which was not present before addition of eserine (CONTROL). The amplitude of the first population spike was not affected. The second population spike was completely reversed by 48 min wash with drug-free buffer (WASH).**

B. **In contrast, the second population spike elicited by the organophosphate, DFP (10 µM), applied for 150 min, was actually increased in amplitude after 90 min wash. The first population spike was unaffected.**

C. **The organophosphate, soman (100 µM), applied for 25 min, also elicited a second population spike which was not completely reversed by 46 min wash. There was no change in the first population spike.**

A second population spike was elicited by concentrations of eserine from 100 nM to 100 µM, DFP from 1 µM to 100 µM, and soman from 1 µM to 100 µM. The second population spike appeared within 20 min of addition of drug and quickly reached its maximum amplitude. None of these concentrations of eserine or soman affected the first population spike. The highest concentration (100 µM) of DFP significantly decreased the amplitude of the first population spike. None of the anticholinesterases, at any concentration tested, affected the antidromic population spike elicited by stimulation of the alveus.

ATROPINE BLOCKS THE EFFECTS OF ACH BUT NOT DFP



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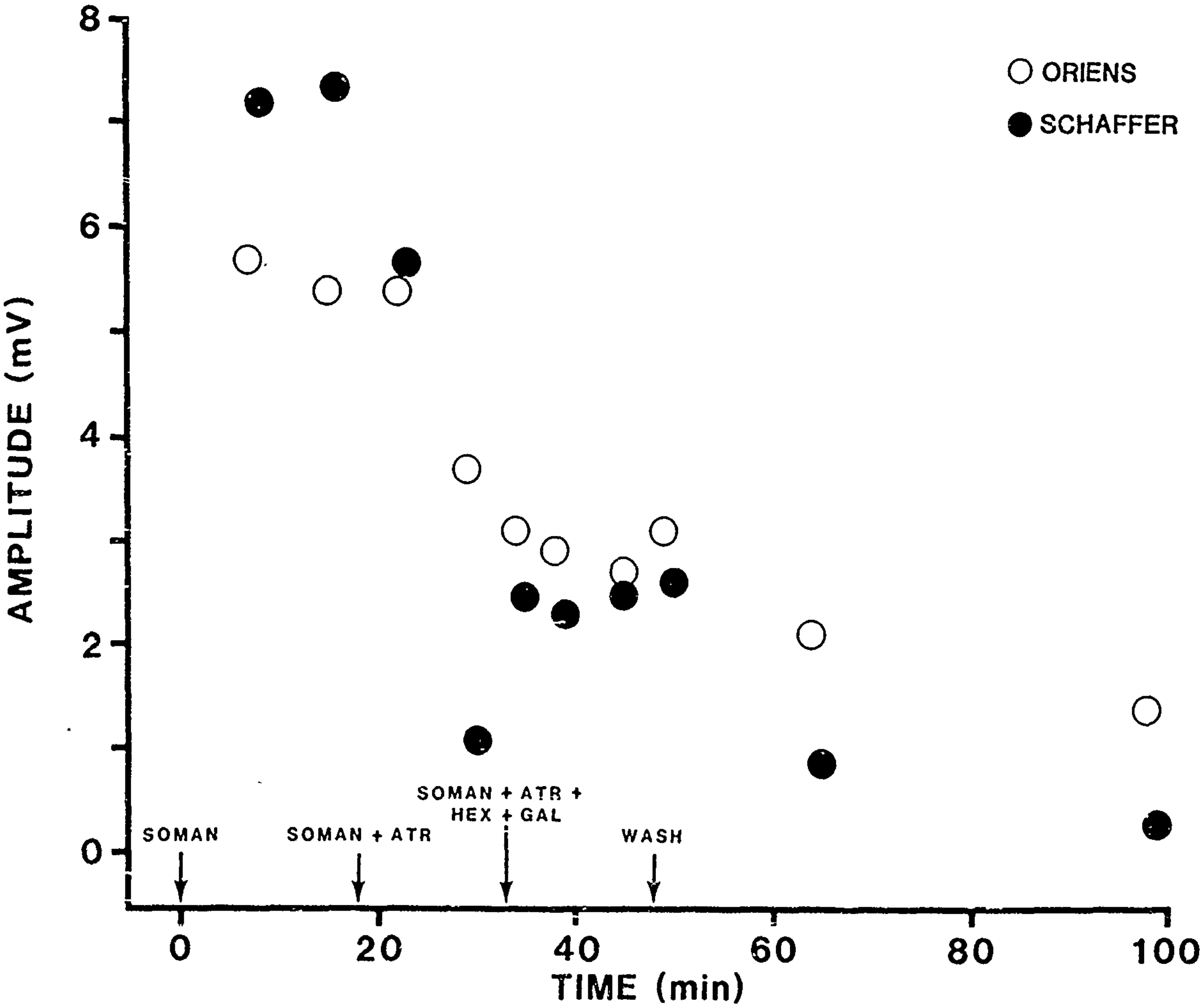
A. The left column (PRE-ACH) shows the response of a slice to stimulation of the Sch (125 µA, 100 usec) just prior to iontophoretic application of ACH (1 M, 10 sec, 150 nA, -25 nA retaining current). The right column (ACH 10S) shows the population spike 10 sec after the onset of the iontophoretic pulse, when the response to ACh was maximal. There was a slight reduction in the amplitude of the first spike, and a second spike was clearly visible.

B. Atropine (1 µM) was bath applied for 10 min. It had no effect on the population spike amplitude. However, atropine competely blocked the excitatory effect of iontophoretically applied ACh.

C. Twenty min after addition of 10 µM DFP in the continued presence of atropine, a large second population spike was visible. Therefore, in additioin to its well known-known anticholinesterase effect, DFP appears to produce a second population spike by a second mechanism.

Atropine (1-10 µM) applied either before (n=3) or after (n=4) exposure to 10 µM DFP did not significantly affect the occurrence or amplitude of the second population spike. The irreversible muscarinic antagonist, quinuclidinyl benzylate (QNB), was also ineffective in preventing or reversing the second population spike induced by DFP (n=5). In addition, experiments with the nicotinic antagonists, gallamine, hexamethonium, and dihydro-ß-erythroidine (all 10 µM), separately, and in combination with atropine, failed to reduce (n=9) the second population spike produced by 10 µM DFP. The nicotinic antagonists and atropine had no significant effect on the amplitude of the or odromic population spike (n=13).

CHOLINERGIC ANTAGONISTS DECREASE, BUT DO NOT REVERSE, THE EFFECT OF SOMAN



Prior to application of soman (first arrow), there was no second population spike. In soman (10 µM), a second population spike developed in response to orthodromic stimulation of both the oriens (OPEN CIRCLES; between the alveus and cell body layer in Figure 1) and the Schaffer collaterals (CLOSED CIRCLES). The amplitude of the second spike was reduced when 10 µM atropine (ATR) was added in the continued presence of soman. Addition of the ganglionic nicotinic antagonist, hexamethonium (HEX; 10 µM), and the neuromuscular nicotinic antagonist, gallamine (GAL; 10 µM), to the bath had no further effect on the second population spike. The amplitude of the second spike was further reduced, but not completely reversed, upon prolonged wash with drug-free buffer. The amplitude of the first spike (not shown) was 9.5 mV (oriens) and 9.2 mV (Schaffer), and was unaffected by any of the treatments.

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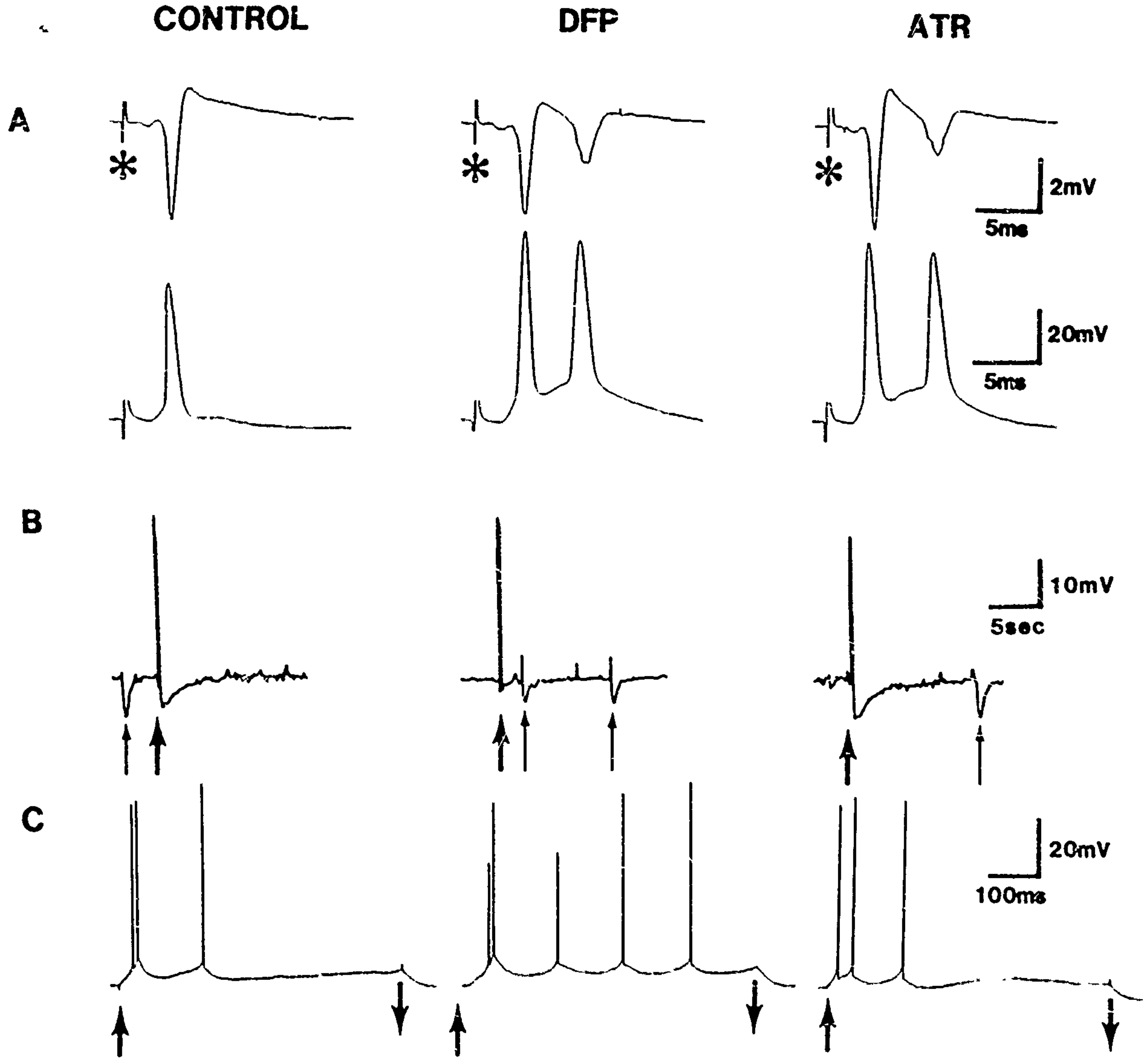
ATROPINE REVERSES INTRACELLULARLY RECORDED CHOLINERGIC EFFECTS, BUT NOT THE SECOND ACTION POTENTIAL ELICITED BY DFP



A. Extracellular (UPPER TRACES) and intracellular (LOWER TRACES) recordings of the response to stimulation of Sch. Before addition of DFP (CONTROL), stimulation elicited only a single population spike and a single intracellular action potential. After 20 min exposure to 10 µM DFP, both the extra- and intracellular traces showed characteristic second spikes. Exposure to atropine (ATR; 10 µM; 20 min) did not reduce the amplitude of the second population spike in the upper trace or eliminate the second action potential in the lower trace.

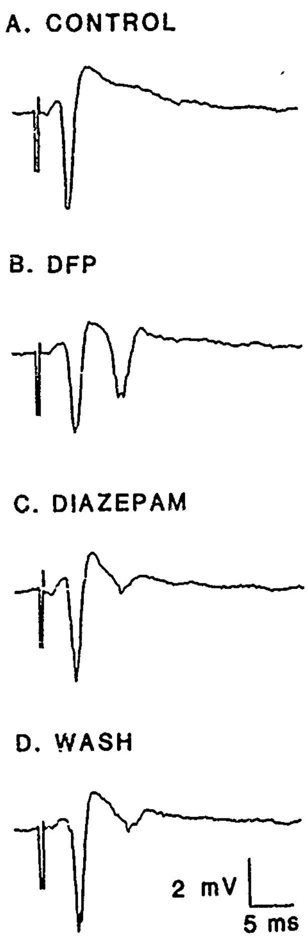
B. Intracellular recording of the afterhyperpolarization following a burst of action potentials (HEAVY ARROW; action potentials truncated) elicited by injection of a depolarizing current pulse (0.5 nA, 100 msec) through the recording electrode. In CONTROL, an afterhyperpolarization immediately followed the action potentials. In the presence of DFP, the afterhyperpolarization was virtually eliminated. Atropine (ATR) restored the afterhyperpolarization.

The small, spontaneous hyperpolarizations (LIGHT ARROWS), which may be spontaneous inhibitory postsynaptic potentials, were also reduced in amplitude, but not frequency, by DFP; their amplitude was restored by atropine.

C. Intracellular recording of accommodation during a burst of action potentials by a depolarizing current pulse (0.2 nA, 600 msec; onset at UPWARD and offset at DOWNWARD ARROW). In CONTROL, only three action potentials fired. In DFP, five action potentials fired, indicating a decrease in accommodation. Atropine (ATR) restored accommodation so that the cell fired only three action potentials, as in the control condition.

All intracellular recordings are from the same cell. It is well established that acetylcholine blocks accommodation and the afterhyperpolarization by a muscarinic action (Cole & Nicoll, J. Physiol. 352:173, 1984). This further supports the conclusion that DFP must be acting through some other mechanism besides inhibition of cholinesterase.

DIAZEPAM DECREASES THE SECOND POPULATION SPIKE IN DFP



A. Orthodromic (Schaffer collateral) stimulation elicited only a single population spike.

B. After exposure to 10 µM DFP for 20 min, stimulation elicited a second population spike.

C. Subsequent exposure to 1 µM diazepam for 35 min markedly reduced the second population spike.

D. Response after 20 min wash with drug-free buffer.

Stimulus strengths were adjusted to produce first population spikes with similar amplitudes. They were (A) 210, (B) 150, (C) 225, (D) 200 µA; duration was 200 µsec for A-D.

CONCLUSIONS

1. At concentrations up to 100 μM, the anticholinesterases, eserine and soman, had no significant effects on the amplitude of the orthodromic population spike in field CA1. In 100 μM DFP, the amplitude of the first population spike was significantly depressed; but lower concentrations did not affect the amplitude of the population spike. Antidromic population spikes were not affected by any concentration of the three anticholinesterases.

2. The major effect of the anticholinesterases was manifested by the appearance of a second population spike. After exposure to eserine, the second population spike was reversed by wash. In contrast, after exposure to DFP or soman, the second spike was not reversed by wash.

3. The muscarinic antagonist, atropine, completely blocked the second population spike elicited during iontophoretic application of acetylcholine. However, muscarinic and nicotinic antagonists were completely ineffective in reversing the second spike elicited by DFP. The second spike elicited by soman was only partially reversed by atropine.

4. Intracellular recordings revealed that atropine completely restored accommodation and the after-hyperpolarization which were blocked by DFP. Thus, atropine was capable of reversing those effects of DFP which could be attributed to accumulation of ACh. Nevertheless, the intracellular correlate of the second population spike (that is, a second action potential) elicited in DFP was not reversed by atropine.

5. Diazepam markedly reduced the second population spike elicited in DFP.

6. Therefore, it appears that the second population spike seen in 10 μM DFP and 10 and 100 μM soman may be partially the result of an interaction at a non-nicotinic, atropine-resistant site. Diazepam produces a striking decrease in the second spike. The mechanism of action of organophosphates and the effectiveness of combinations of drugs to block their effects are under investigation.

IN VIVO HIPPOCAMPAL pO2 TRANSIENTS DURING BICYCLIC ORGANOPHOSPHATE-INDUCED SEIZURES IN THE RAT

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INTRODUCTION

THE HIPPOCAMPUS IS ONE OF SEVERAL AREAS WHICH SHOWS PARTICULAR VULNERABILITY TO ANOXIC EPISODES. HIPPOCAMPAL SCLEROSIS IN HUMAN TEMPORAL LOBE EPILEPTICS IS ANOTHER MANIFESTATION OF HIPPOCAMPAL VULNERABILITY, ALTHOUGH IT IS NOT NECESSARILY RELATED TO ANOXIA. HOWEVER, THE POSSIBILITY THAT HYPOXIA IS A FACTOR IN HIPPOCAMPAL CELL DEATH IS UNDERSCORED BY THE UNUSUAL FINDINGS OF ACKERMAN ET AL. AND YAN ET AL. THAT DURING KINDLED OR BICUCULLINE SEIZURES IN RATS, HIPPOCAMPAL BLOOD FLOW DECREASES WHILE 2DG UPTAKE INCREASES. BY CONTRAST, MOST STUDIES OF CEREBRAL BLOOD FLOW HAVE SHOWN GLOBAL INCREASES DURING SEIZURES. FURTHERMORE, LYNCH AND JACKSON FOUND THAT THE PO_2 OF THE AMYGDALA CONSISTENTLY INCREASED DURING KINDLED SEIZURES. THUS THE QUESTION AROSE, "DOES THE HIPPOCAMPUS BECOME HYPOXIC, AND IS ITS PO_2 OBVIOUSLY DIFFERENT FROM THAT OF OTHER AREAS DURING A GIVEN SEIZURE?"

WE CHOSE TO MONITOR IN VIVO BRAIN PO_2 IN HIPPOCAMPUS, AMYGDALA AND FRONTAL CORTEX DURING SEIZURES INDUCED BY THE BICYCLIC ORGANOPHOSPHATE AND ANTI-GABA AGENT, ETHYL-PTBO. ETHYL-PTBO CAUSES SEIZURES AND DEATH WITHIN MINUTES IN RATS AND IS A POTENTIAL HAZARD SINCE IT IS THE CHIEF COMBUSTION PRODUCT IN THE BURNING OF FIRE-RETARDED PLASTICS.

METHODS

MALE SPRAGUE-DAWLEY RATS UNDERWENT STEREOTAXIC IMPLANTATION OF A PLATINUM PO_2 ELECTRODE IN EITHER DORSAL OR TEMPORAL HIPPOCAMPUS, AMYGDALA OR FRONTAL CORTEX. PO_2 ELECTRODES WERE 100 MICRONS IN DIAMETER AND THE WORKING SURFACE WAS COATED WITH RHOPLEX. A STANDARD DENTAL CEMENT HEAD MOUNT AND CABLE SYSTEM WAS USED, ALLOWING SECURE RECORDING OF PO_2 AND CORTICAL EEG FROM THE FREE RANGING ANIMAL. CURRENT TO VOLTAGE CONVERSION WAS USED TO OBTAIN A CHART TRACING PROPORTIONAL TO BRAIN PO_2.

ONE WEEK FOLLOWING SURGERY EACH RAT WAS GIVEN AN I.P., LD_{50} INJECTION OF ETHYL-PTBO, .77mG/Kg. RECORDS OF BRAIN PO_2 AND CORTICAL EEG WERE OBTAINED DURING THE CONVULSIONS WHICH FOLLOWED.

RESULTS

1) DURING ETHYL-PTBO CONVULSIONS THE DORSAL AND TEMPORAL HIPPOCAMPI INCURRED TRANSIENT HYPOXIA OF SIGNIFICANT DEGREE, BUT SO ALSO DID THE AMYGDALA AND THE FRONTAL CORTEX.

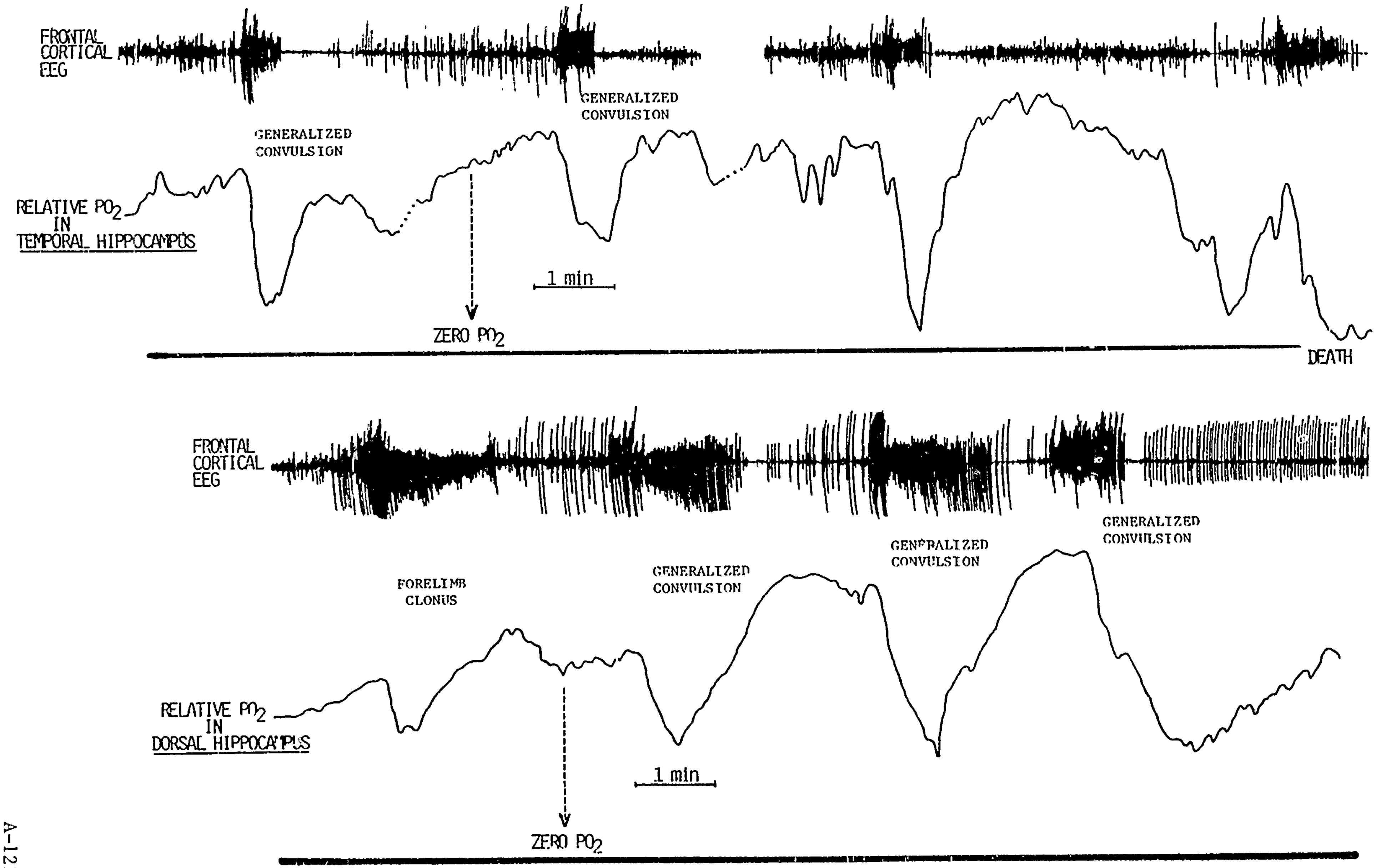
2) FROM FIRST REACTION TO LAST, THE TYPICAL ETHYL-PTBO EPISODE HAS THE FOLLOWING SEQUENCE:

1. PRONATION
2. MYOCLONIC JERKS
3. JAW CLONUS, HEAD NODS, UNILATERAL FORELIMB CLONUS
4. BILATERAL FORELIMB CLONUS
5. GENERALIZED TONIC-CLONIC CONVULSION
6. STATUS

3) THE TYPICAL PO_2 TRANSIENT IS A SLIGHT HYPEROXIA ASSOCIATED WITH EARLY CLONIC COMPONENTS...FOLLOWED BY A 36% DECREASE IN PO_2 ASSOCIATED WITH THE CLONIC-TO-TONIC TRANSITION AND RUNNING ITS COURSE IN 40 SECONDS....ENDING WITH A 40% OVERSHOOT OF BASELINE PO_2 ASSOCIATED WITH THE LATE TONIC-TO-POSTICTAL TRANSITION AND RUNNING ITS COURSE IN 2 MINUTES.

4) THE MEAN HYPOXIC AND POSTICTAL HYPEROXIC LEVELS MEASURED IN DORSAL HIPPOCAMPUS WERE NOT SIGNIFICANTLY DIFFERENT FROM THOSE MEASURED IN TEMPORAL HIPPOCAMPUS, NOR WAS THE MEAN AMYGDALA HYPOXIA SIGNIFICANTLY DIFFERENT FROM THAT IN EITHER HIPPOCAMPAL COMPONENT.

	% HYPOXIA	% POSTICTAL HYPEROXIA	SEIZURES	RATS
DORSAL	36.7 +11.3	34.4 +28	22	7
TEMPORAL	36.0 +18.9	41.3 +33	19	6
AMYGDALA	45.3 +18.2	—	20	4


FRONTAL
CORTICAL
EEG
GENERALIZED
CONVULSION
GENERALIZED
CONVULSION
RELATIVE PO_2
IN
TEMPORAL HIPPOCAMPUS
1 min
ZERO PO_2
DEATH
FRONTAL
CORTICAL
EEG
FORELIMB
CLONUS
GENERALIZED
CONVULSION
GENERALIZED
CONVULSION
GENERALIZED
CONVULSION
RELATIVE PO_2
IN
DORSAL HIPPOCAMPUS
1 min
ZERO PO_2

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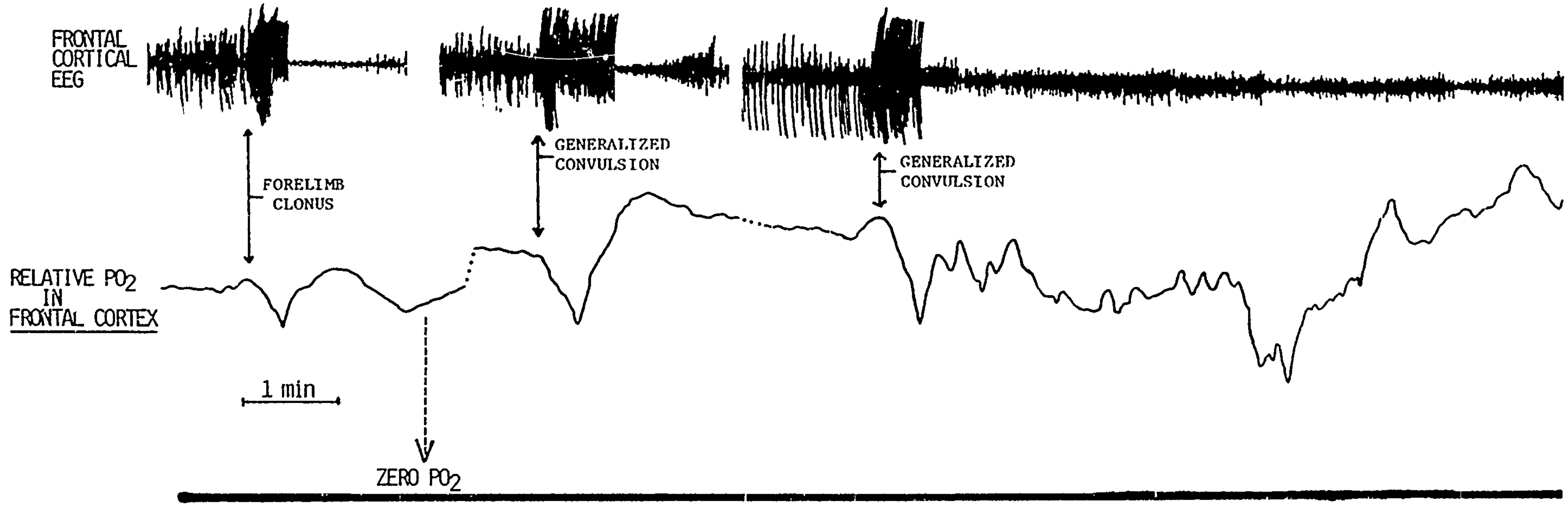
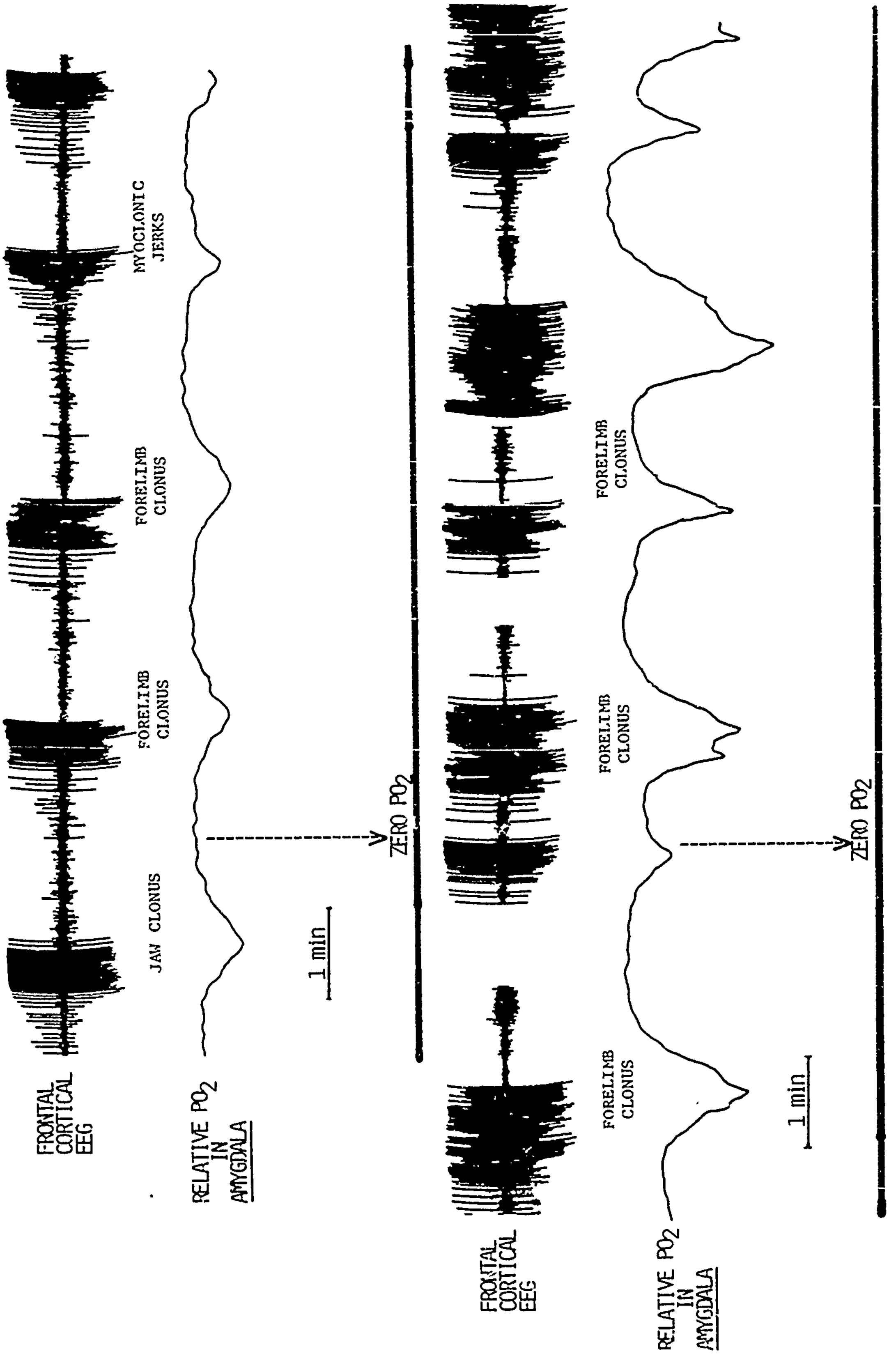
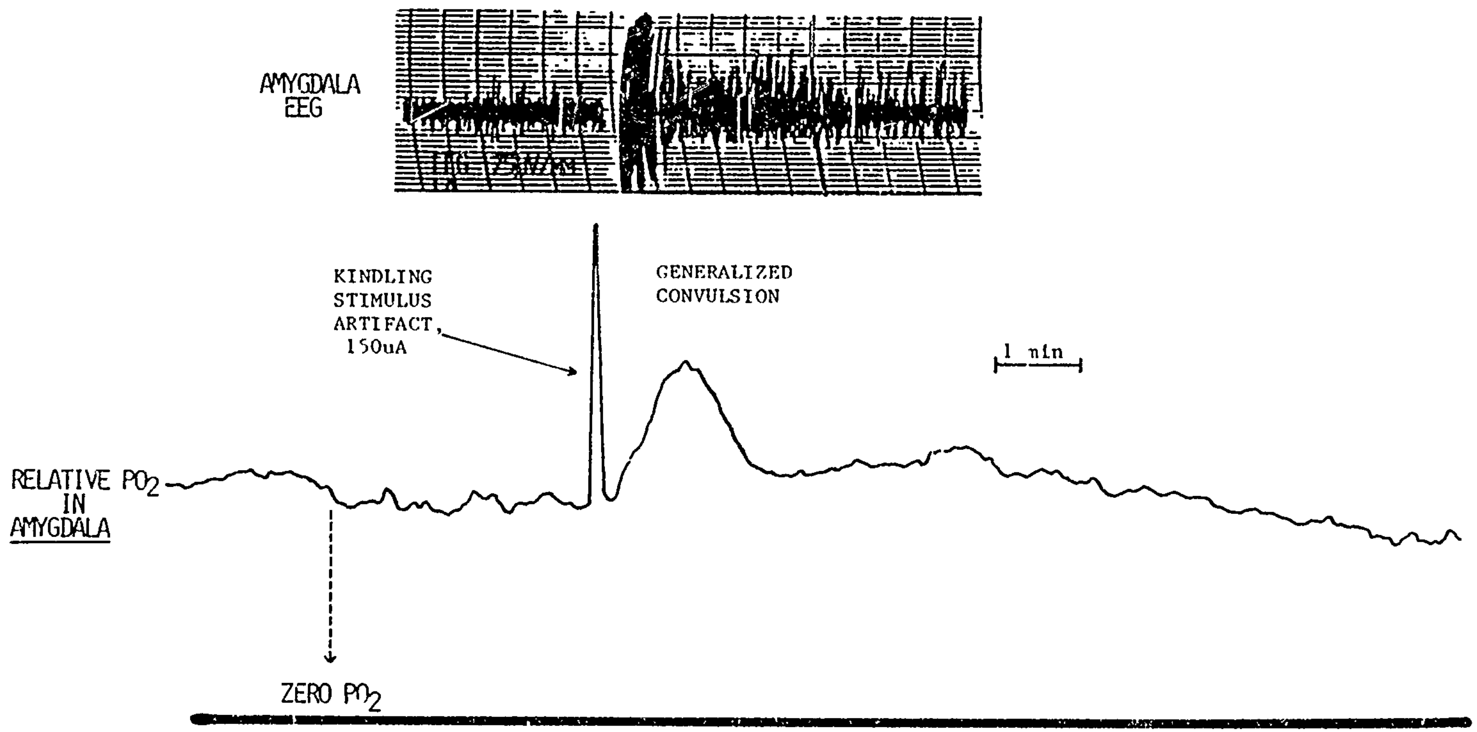
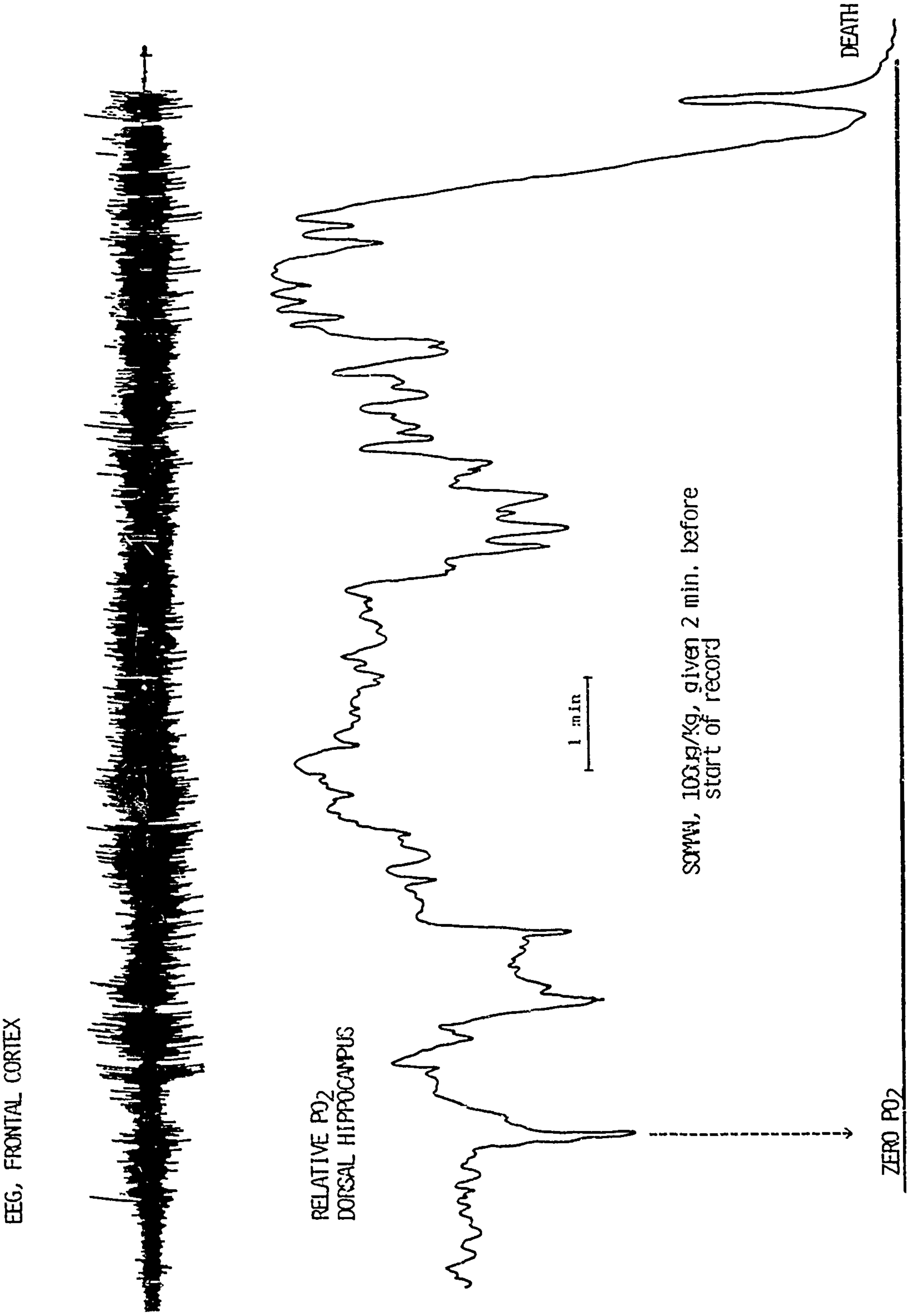

FRONTAL CORTICAL EEG
FORELIMB CLONUS
GENERALIZED CONVULSION
GENERALIZED CONVULSION
RELATIVE PO_2 IN FRONTAL CORTEX
1 min
ZERO PO_2



FRONTAL CORTICAL EEG
RELATIVE PO_2 IN AMYGDALA
JAW CLONUS
FORELIMB CLONUS
FORELIMB CLONUS
MYOCLONIC JERKS
1 min
ZERO PO_2
FRONTAL CORTICAL EEG
RELATIVE PO_2 IN AMYGDALA
FORELIMB CLONUS
FORELIMB CLONUS
FORELIMB CLONUS
1 min
ZERO PO_2



IN THE AMYGDALA, THE KINDLING PO_2 TRANSIENT IS DIFFERENT FROM THE ETHYL-PTBO PO_2 TRANSIENT


EEG, FRONTAL CORTEX
RELATIVE PO_2
DORSAL HIPPOCAMPUS
1 min
SOMAN, 100µg/Kg, given 2 min. before
start of record
DEATH
ZERO PO_2

CONCLUSIONS

1. HYPOXIA DURING ETHYL-PTBO CONVULSIONS IS PRIMARLILY ASSOCIATED WITH A GENERALIZED AND SEVERELY TONIC ONSET. RESPIRATORY EMBARRASSMENT IS THE PROBABLE CAUSE OF THIS HYPOXIA.

2. TRANSIENT HYPOXIA IN ETHYL-PTBO CONVULSIONS SEEMS TO BE A GLOBAL RESPONSE, I.E. NOT SPECIFIC TO THE HIPPOCAMPUS. IF HYPOXIA/ANOXIA IS A FACTOR IN SEIZURE-RELATED HIPPOCAMPAL CELL LOSS, IT IS PROBABLY DUE NOT TO SEVERE HYPOXIA PER SE, BUT TO A SELECTIVE VULNERABILITY TO ANY CHANGES IN PO_2, pH, CALCIUM CONCENTRATION OR SUBSTRATE DELIVERY. THE DECREASE IN HIPPOCAMPAL BLOOD FLOW OBSERVED BY ACKERMAN ET AL. AND YAN ET AL. MAY COMPOUND THESE FACTORS.

3. SINCE KINDLED CONVULSIONS ARE PRIMARILY CLONIC, INVOLVING LITTLE OR NO RESPIRATORY EMBARRASSMENT, THE LIKELY INDUCTION OF HYPEREMIA IN THE AMYGDALA IS MANIFESTED AS AN INCREASE IN AMYGDALA PO_2. SUCH AN EFFE IN ETHYL-PTBO CONVULSIONS IS PROBABLY MASKED BY RESPIRATORY FAILURE.

INTERACTION OF CAGE CONVULSANTS WITH GABA-GATED CHLORIDE CHANNELS IN CULTURED CEREBRAL NEURONS

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ABSTRACT

The cage convulsant t-butylbicyclophosphorothionate (TBPS) is one of the most potent seizure-inducing organophosphate compounds known (LD_{50} = 40 μg/kg) but it is not an inhibitor of acetylcholinesterase [Milbrath, D.S. et al. (1979) Toxicol. Appl. Pharmacol. 47, 287-293]. The role of TBPS as an antagonist of γ-aminobutyric acid (GABA) has been studied with primary cultures of neurons from the chick embryo cerebrum. The activity of Cl^- channels has been analyzed by the transport of $^{36}Cl^-$ into neuronal monolayers which is dependent upon the addition of GABA ($K_{0.5}$ = 1.5 μM). Kinetic studies of GABA-gated $^{36}Cl^-$ flux reveal that TBPS is an inhibitor (IC_{50} = 280 nM) whose action is noncompetitive (K_i = 140 nM) with respect to GABA. In this assay, TBPS is 100 times more potent than the less toxic ethyl derivative (ethyl bicyclophosphorothionate) and 10 times more potent than picrotoxin. TBPS also blocked the basal $^{36}Cl^-$ flux observed in the absence of GABA, although higher concentrations were required (IC_{50} = 7.5 μM).

The direct binding of [^{35}S]TBPS to isolated neuronal membrane vesicles was determined using a filtration assay. [^{35}S]TBPS was specifically bound to neuronal membranes and could be readily displaced by picrotoxinin or unlabeled TBPS. Scatchard plots of these binding data were curvilinear but could be resolved by non-linear regression analysis into two components. These represent two membrane sites which differ in affinity for TBPS (K_{d1} = 2.1 nM; K_{d2} = 270 nM). The binding constant for the site of lower affinity agrees well with the IC_{50} and K_i values observed for TBPS inhibition of GABA-gated Cl^- flux. The high affinity site for TBPS binding thus appears to be less relevant to the function of GABA channels. The binding of TBPS to membranes was completely dependent upon the addition of conductive anions which were effective in the order: $Br^- > SCN^- > Cl^- > I^- > F^- >$ gluconate. This rank order is the same as that observed for GABA-gated anion conductance [Araki, T., et al. (1961) J. Physiol. (London) 159, 410-435]. These data suggest that TBPS exerts its convulsive effect via blockade of Cl^- channels. This work supported in part by US Army Medical Research Development Command under Contract DAMD17-84-C-4102.

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INTRODUCTION

The cage convulsant, TBPS (Fig. 1) is one of the most potent seizure-inducing organophosphate compounds known (LD_{50} = 50 μg/kg i.p. in mice). However, it is not an inhibitor of acetylcholinesterase (Milbrath et al., 1979). It has been suggested that the neurotoxic effects of cage convulsants are produced by an antagonism of GABAergic neurotransmission (Bowery et al., 1976).

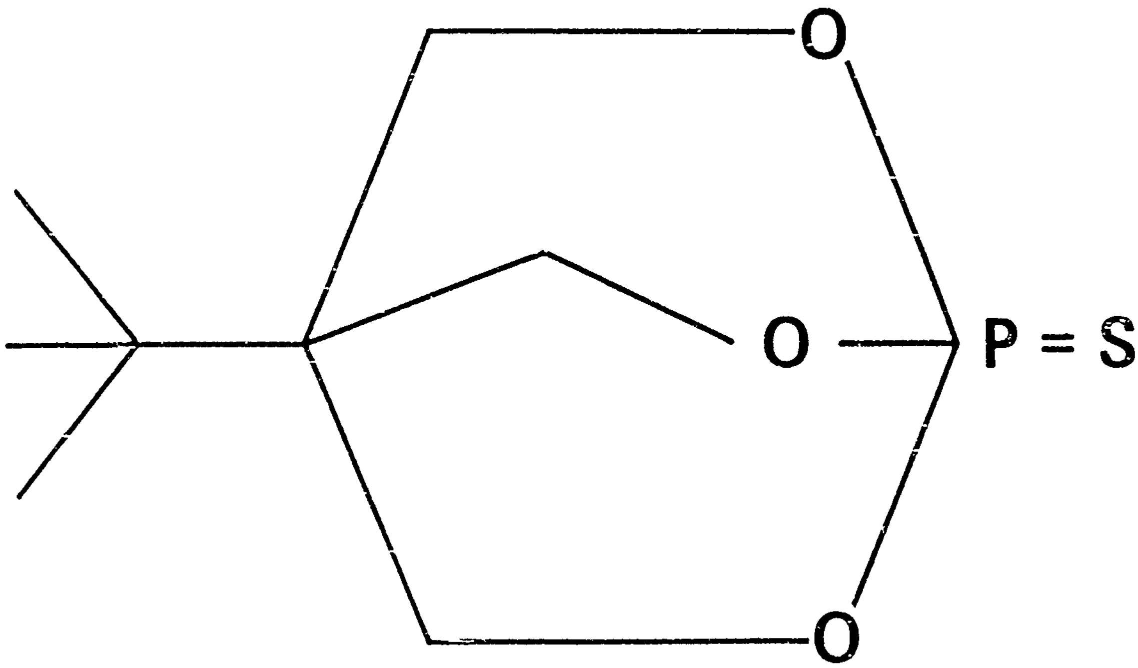
Our laboratory has been investigating the interaction of cage convulsants with cultured cerebral neurons from the chick embryo, a preparation enriched in GABAergic cells (Thampy et al., 1983). These compounds have been shown to block the $^{36}Cl^-$ uptake into neurons which is mediated by GABA channels (Thampy and Barnes, 1984). Here we report studies of the interaction of TBPS with Cl^- channels in cerebral neurons by means of $^{36}Cl^-$ flux kinetics (Thampy and Barnes, 1984) and by direct binding of [^{35}S]TBPS to isolated membranes (Squires et al., 1983).

Table I

IC_{50} Values for Inhibition of Cl^- Flux by GABA Antagonists

Antagonist	IC_{50} Value	
	Basal flux	GABA-gated
	μM	μM
TBPS	7.8	0.28
EBPS	19	25
Picrotoxin	>50	5.0
Bicuculline	>50	3.5

The IC_{50} values shown were determined from plots such as that shown in Fig. 4.



t–Butylbicyclophos–
phorothionate (TBPS)

Fig. 1 TBPS Structure

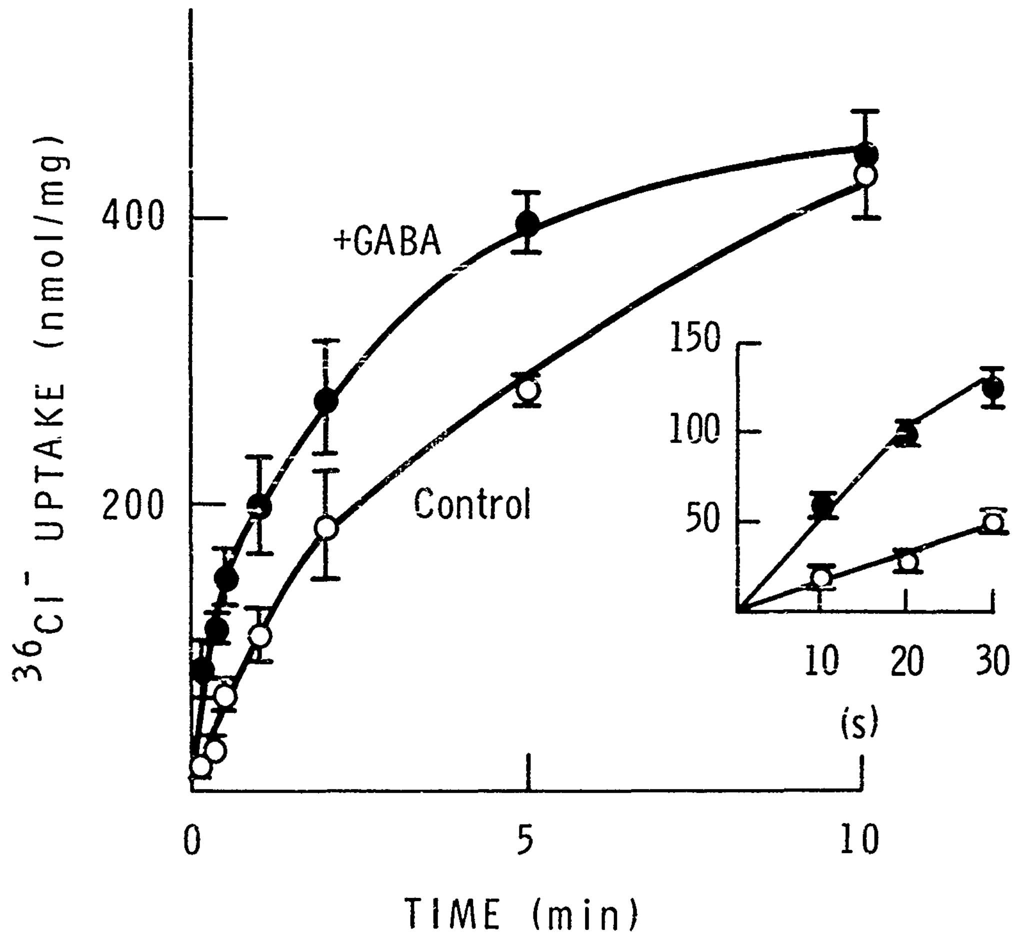


Fig. 2. $^{36}Cl^-$ Uptake by Cultured Cerebral Neurons. Neuronal monolayers on plastic coverslips were incubated in HEPES buffered saline containing 40 mM K^+ and 138 mM $^{36}Cl^-$ for the times shown. Where indicated (●), 50 μM GABA was also present. Uptake was terminated by washing in ice cold saline. Results are expressed per mg of cellular protein.

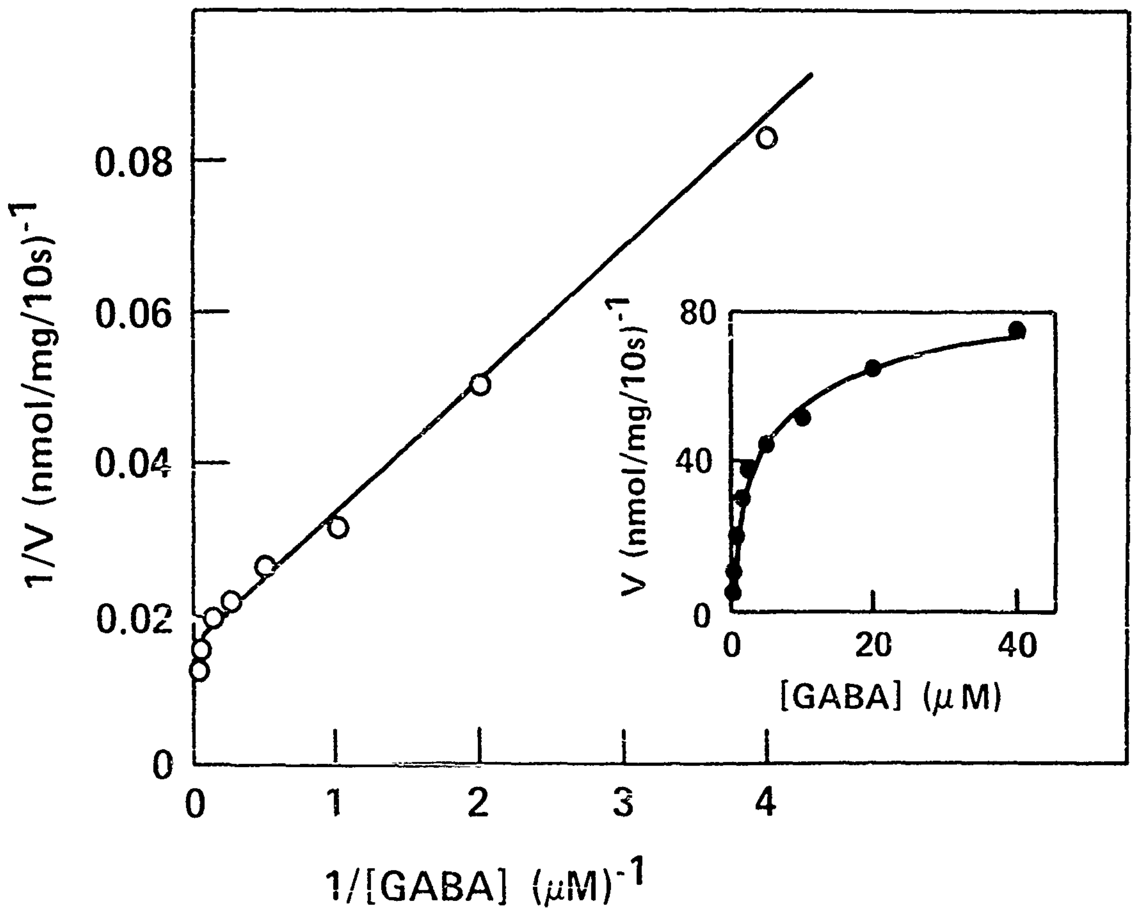


Fig. 3. GABA dose dependence for Cl^- uptake. Assays were carried out as indicated in Fig. 2 except that incubations were terminated after 10 s and GABA concentrations were as shown. All data were corrected by subtraction of values for the basal Cl^- uptake which occurred in the absence of GABA. The least squares line shown had r = 0.99. The calculated $K_{0.5}$ for GABA was 1.3 μM.

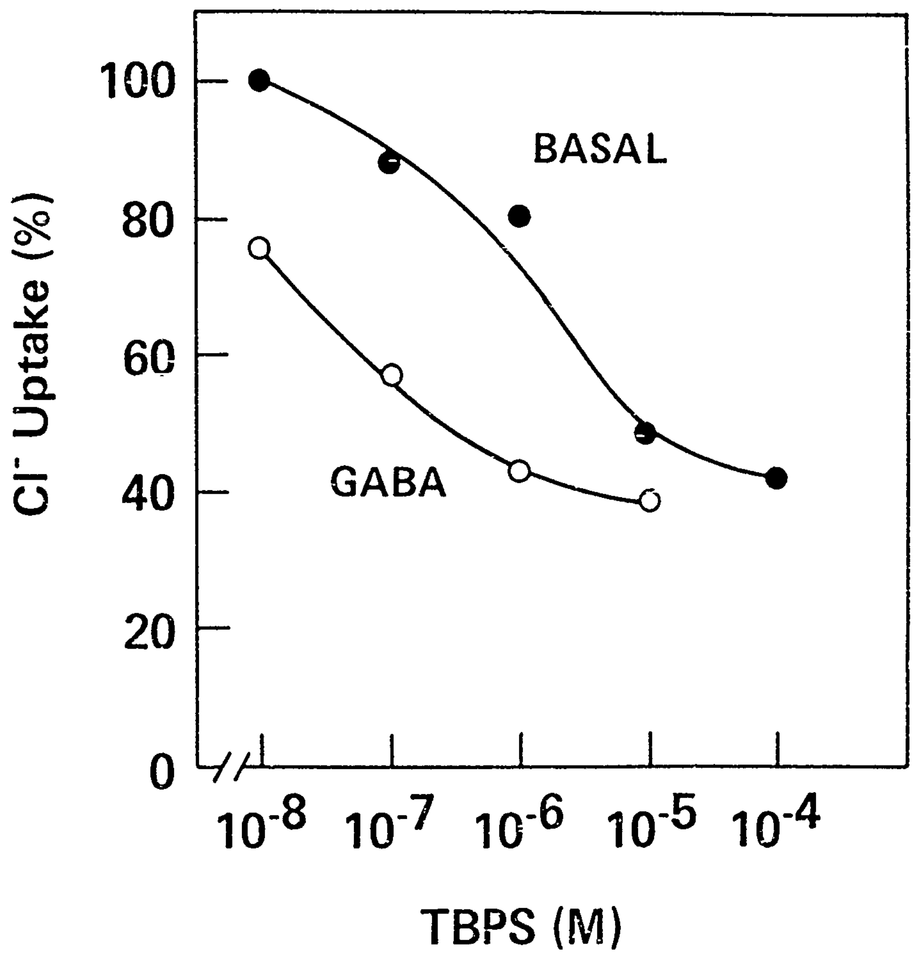


Fig. 4. TBPS dose dependent inhibition of Cl^- flux. Assays were carried out as in Figs. 2 and 3 except that TBPS was added at the concentrations shown.

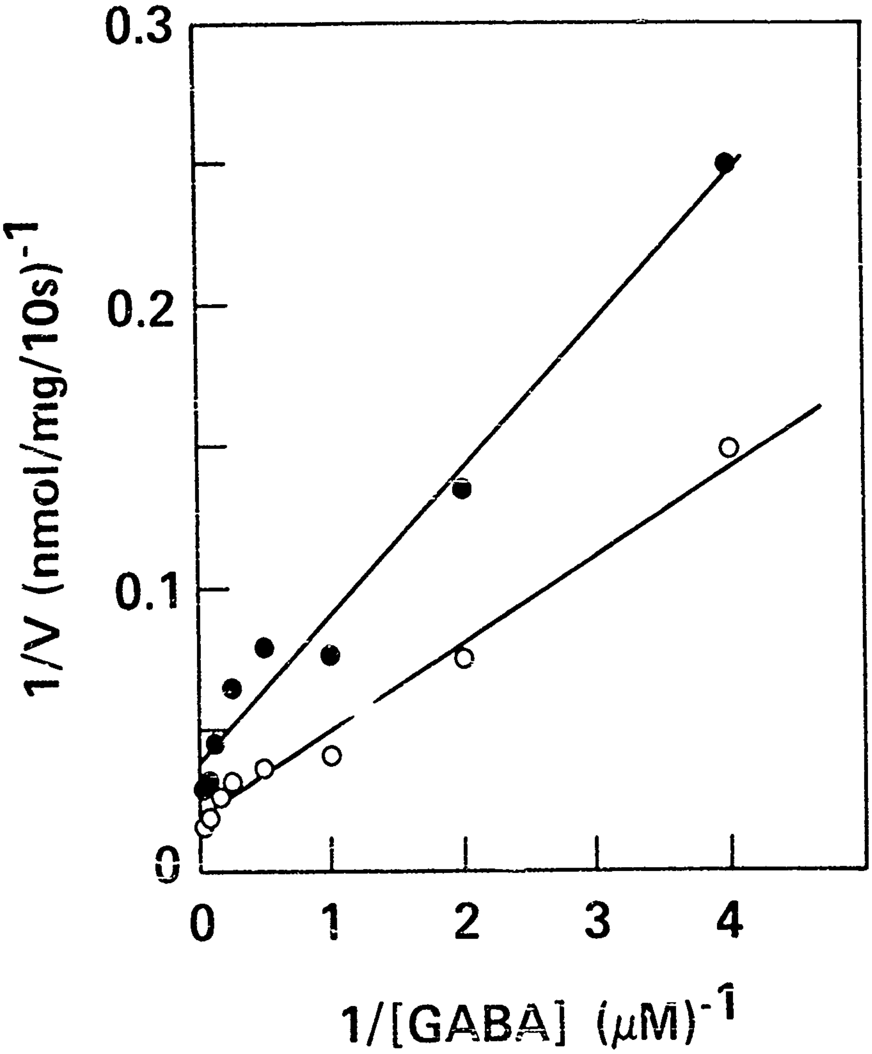


Fig. 5. TBPS inhibition kinetics for GABA-dependent Cl^- uptake. Assays were carried out as in Fig. 3 except that 0.2 μM TBPS was added where indicated (●). The inhibition by TBPS is apparently noncompetitive with a K_i value of 200 nM. The linear regression lines shown had r values of 0.98 (O) and 0.97 (●).

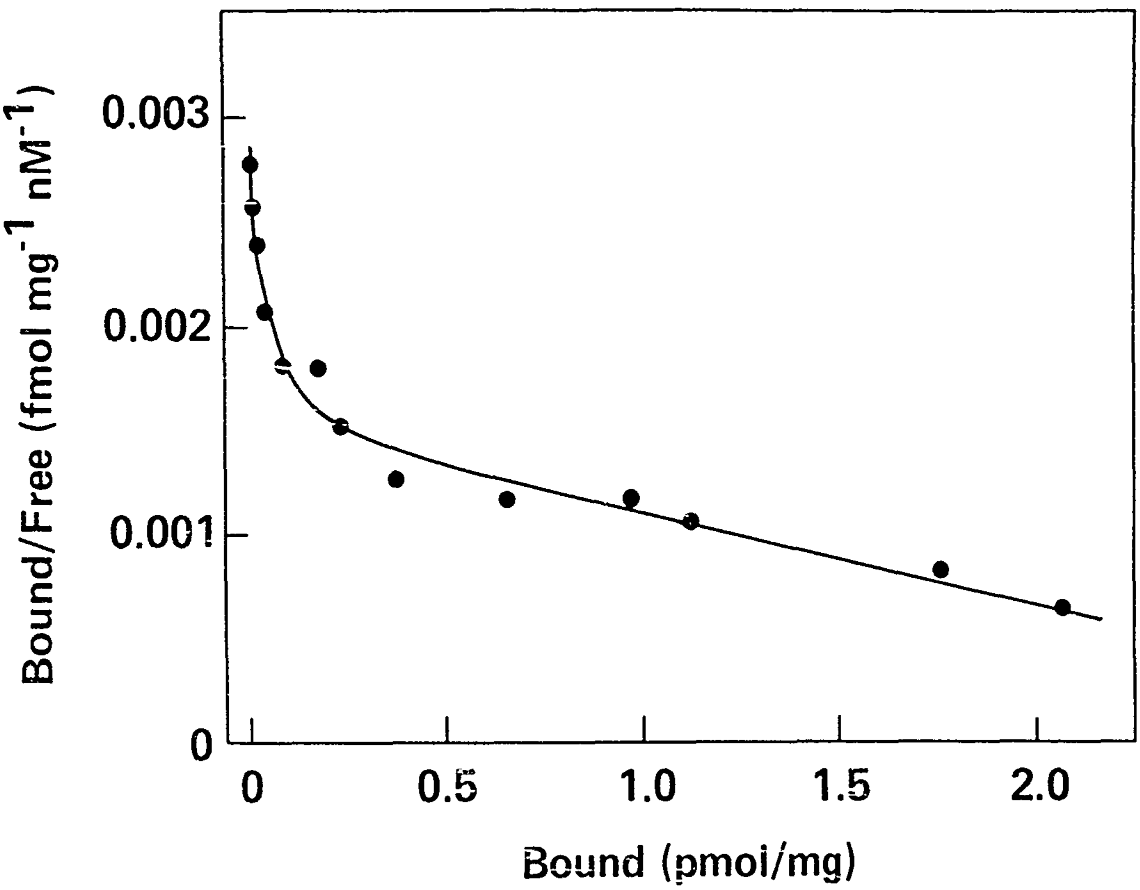


Fig. 6. Scatchard analysis of specific binding of [^{35}S]TBPS to membranes isolated from cerebral neurons. Membranes from neurons (100 µg) were incubated with 250 mM NaBr in 20 mM HEPES Tris (pH 7.4) containing 0.2 to 500 nM [^{35}S]TBPS. After 90 min at 25°, the mixtures were filtered. Binding data were corrected for nonspecific binding by subtraction of values from assays containing 100 µM picrotoxinin. The solid line is the best computer fit based on two binding sites (LIGAND program).

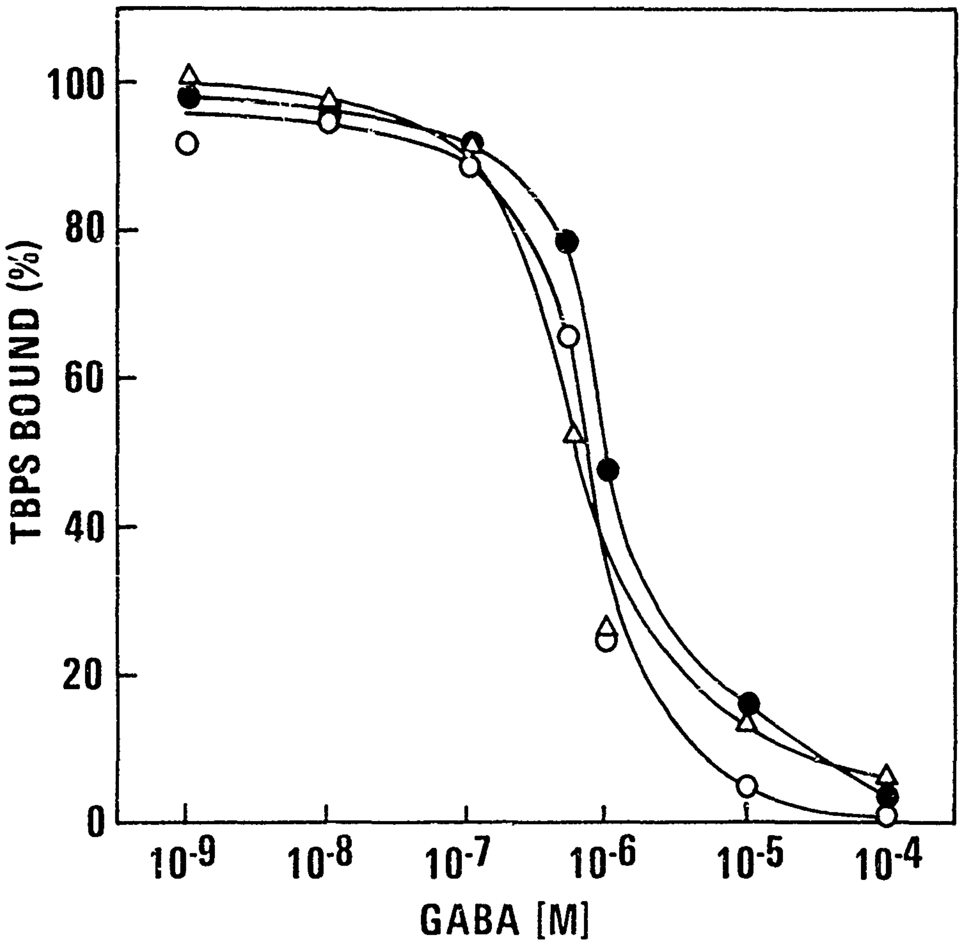


Fig. 7. Displacement of TBPS from neural membranes by GABA. Assays were carried out as in Fig. 6 except that the TBPS concentration was 2 nM and GABA was added at the concentrations shown. Membranes from embryonic chick brain (O), adult chicken brain (●), or from cultured neurons (△). The IC_{50} value for GABA displacement was 0.6 μM for neuronal membranes.

Table II

Parameters for $[^{35}S]$TBPS Binding

Source	$K_d(1)$	$K_d(2)$	$B_{max}(1)$	$B_{max}(2)$
	nM	nM	pmol/mg	pmol/mg
Neurons in Culture	3.12	273	0.031	3.9
Embryonic Chick Brain	1.48	159	0.054	7.0
Adult Chicken Brain	1.39	166	0.111	17.6
Adult Rat Brain	1.15	237	0.085	16.9

K_d and B_{max} values were calculated from plots as in Fig. 6 using the LIGAND program.

Table III

Effects of Anions on TBPS Binding

Anion (50 mM)	TBPS Bound
	fmol/mg
Br^-	90.0 ± 4.3
SCN^-	88.1 ± 2.7
Cl^-	66.9 ± 1.2
NO_3^-	65.8 ± 4.3
I^-	40.7 ± 2.5
F^-	29.1 ± 0.4
SO_4^{2-}	28.9 ± 2.8
Gluconate	21.3 ± 2.4
None	2.4 ± 0.9

TBPS specific binding was determined as in Fig. 6 except that 2 nM TBPS was used and the Na^+ salt indicated replaced 250 nM NaBr.

CONCLUSIONS

1. TBPS is a noncompetitive inhibitor of GABA-gated Cl^- flux in neurons.

2. For blockade of the GABA channel, TBPS is 100 times more potent than the corresponding ethyl derivative (EBPS). This is consistent with the 20-fold differences in LD_{50} (Milbrath et al., 1979).

3. TBPS binds to isolated neuronal membranes at two sites which differ in affinity. The $K_{d(2)}$ value for the site of lower affinity (270 nM) agrees well with the K_i value for inhibition of GABA-gated Cl^- flux (200 nM). However, the site of high affinity binding appears less relevant to inhibition of GABA channels.

4. The $K_{0.5}$ for GABA-gating of the Cl^- flux (1.3 μM) is in reasonable agreement with the IC_{50} value for GABA displacement of TBPS binding (0.6 μM).

5. The rank order for anions as enhancers of TBPS binding to neuronal membranes fits well with the permeability sequence for GABA-channels (Araki et al., 1961).

6. The data indicate that TBPS exerts its convulsive effect via blockade of GABA-gated Cl^- channels, perhaps by interaction with their anion selectivity filter.

ATROPINE OR BENACTYZINE MODIFIES LOCAL CEREBRAL GLUCOSE USE IN SOMAN-INDUCED SEIZURES AND SUBSEQUENT BRAIN DAMAGE

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INTRODUCTION

SOMAN (O,1,2,2-TRIMETHYLPROPYL METHYLPHOSPHONOFLUORIDATE) AT NEAR-LETHAL DOSES PRODUCES REPETITIVE CONVULSIONS THAT LAST FOR SEVERAL HOURS (CLEMENT AND LEE, 1980; MCDONOUGH ET AL., 1983). LOCAL CEREBRAL GLUCOSE UTILIZATION (LCGU) INCREASES (2-5 FOLD) IN MANY BRAIN STRUCTURES DURING THE SEIZURE PHASE (MCDONOUGH ET AL., 1983). THIS REFLECTS THE INCREASED NEURONAL ACTIVITY ASSOCIATED WITH OVERT CONVULSIONS. TWENTY-FOUR TO 72 HR POST SOMAN EXPOSURE CONSPICUOUS BRAIN DAMAGE IS NOTED IN SEVERAL STRUCTURES; DAMAGE IS MOST PROMINENT IN THE PIRIFORM/ENTORHINAL CORTEX, AMYGDALA AND DORSAL THALAMUS. DURING THE PATHOLOGY PHASE, LCGU IS REDUCED (RANGING FROM 10-80% OF CONTROL) IN MOST BRAIN STRUCTURES. THIS REDUCTION IN LCGU REFLECTS BOTH POST SEIZURE DEPRESSION OF BRAIN ACTIVITY AS WELL AS DECREASED ACTIVITY DUE TO DAMAGE IN SELECTED REGIONS OF THE BRAIN (PAZDERNIK ET AL., 1985). THE PURPOSE OF THIS STUDY WAS TO DETERMINE LCGU DURING THE SEIZURE PHASE (15 MIN POST SOMAN EXPOSURE) AND THE PATHOLOGY PHASE (72 HR POST SOMAN EXPOSURE) WHEN RATS WERE PRETREATED WITH ATROPINE OR BENACTYZINE PRIOR TO SOMAN EXPOSURE.

MATERIALS AND METHODS

MALE WISTAR RATS (250-300 G; 3-6/GROUP; CHARLES RIVER BREEDING LABORATORIES, MA) WERE PRETREATED (I.M.) WITH SALINE, ATROPINE SULFATE (3.2 OR 10 MG/KG) OR BENACTYZINE HYDROCHLORIDE (1.0 OR 3.2 MG/KG) 10 MIN PRIOR TO INJECTION (S.C.) OF SOMAN (0.9 LD_{50}). LCGU WAS DETERMINED IN 42 BRAIN REGIONS BY THE QUANTITATIVE AUTORADIOGRAPHIC 2-(^{14}C)-DEOXYGLUCOSE (2-DG METHOD (SOKOLOFF ET AL., 1977) DURING THE SEIZURE PHASE (15 MIN POST SOMAN) OR THE PATHOLOGY PHASE (72 HR POST SOMAN). DETAILS OF THE PROCEDURE HAVE BEEN PREVIOUSLY DESCRIBED (PAZDERNIK ET AL., 1983); THE 2-DG LABELING PERIOD WAS 45 MIN.

EXPERIMENTAL DESIGN

		Seizure Phase	
Group	time/-10 min	0	+ 15 min
I	SAL	SAL	2-DG
II	SAL	SOM	2-DG
III	Atr (3.2 mg/kg)	SOM	2-DG
IV	Atr (10 mg/kg)	SOM	2-DG
V	Atr (10 mg/kg)	SAL	2-DG
VI	Bz (1.0 mg/kg)	SOM	2-DG
VII	Bz (3.2 mg/kg)	SOM	2-DG
VIII	Bz (3.2 mg/kg)	SAL	2-DG

		Pathology Phase	
Group	time/-10 min	0	+ 72 hr
I	SAL	SAL	2-DG
II	SAL	SOM	2-DG
III	Atr (3.2 mg/kg)	SOM	2-DG
IV	Atr (10 mg/kg)	SOM	2-DG
V	Atr (10 mg/kg)	SAL	2-DG
VI	Bz (1.0 mg/kg)	SOM	2-DG
VII	Bz (3.2 mg/kg)	SOM	2-DG
VIII	Bz (3.2 mg/kg)	SAL	2-DG

SAL = Saline; SOM = Soman; 2-DG = 2-(^{14}C)-deoxyglucose;
Atr = Atropine; Bz = Benactyzine

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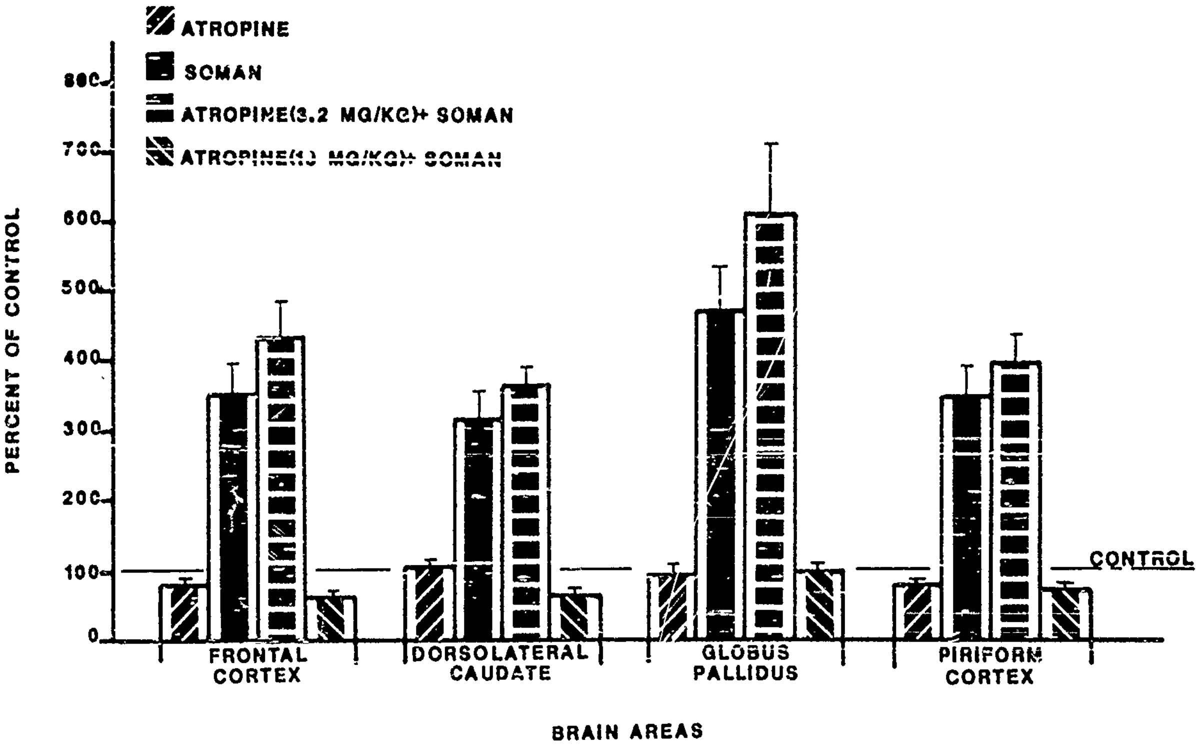


FIGURE 1A. REPRESENTATIVE AUTORADIOGRAPHS OF FRONTAL BRAIN SECTIONS SHOWING LOCAL CEREBRAL GLUCOSE UTILIZATION DURING THE SEIZURE PHASE. RATS WERE TREATED AS DESCRIBED IN THE EXPERIMENTAL DESIGN SECTION; ATROPINE WAS THE PRETREATMENT AGENT. EACH BAR REPRESENTS LOCAL CEREBRAL GLUCOSE UTILIZATION OF THE INDICATED STRUCTURE EXPRESSED AS PERCENT OF VEHICLE CONTROL FOR THE DESIGNATED TREATMENT.

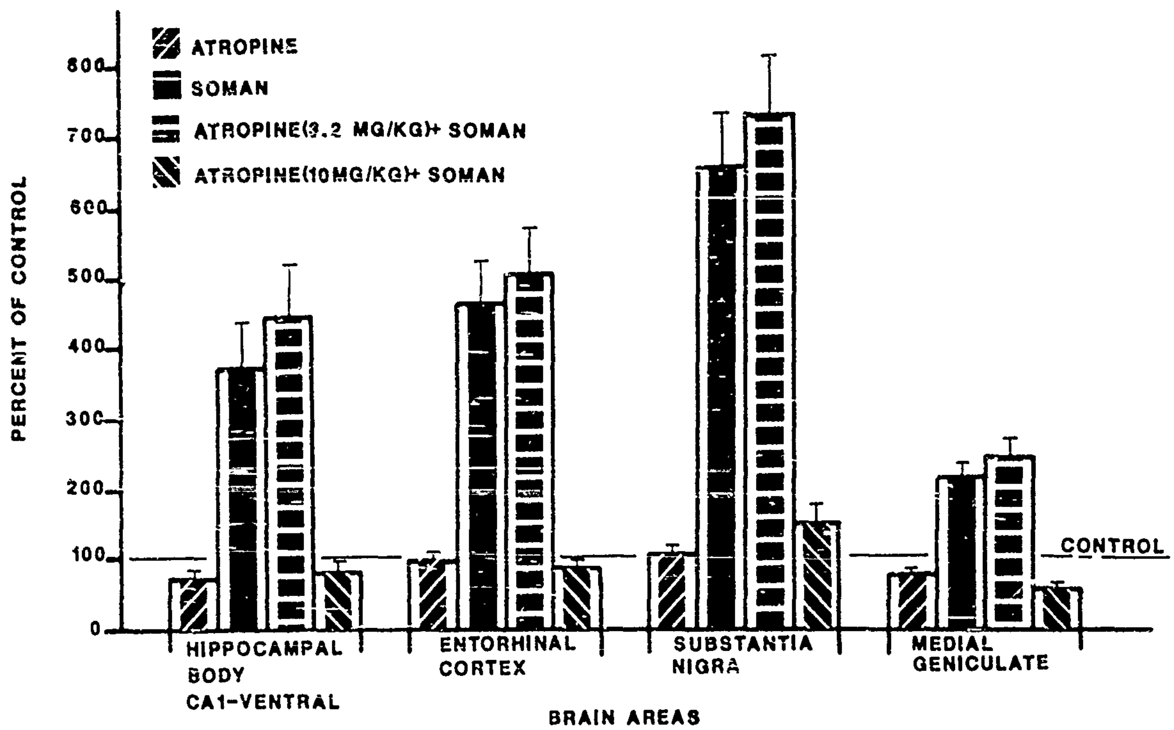


FIGURE 1B. REPRESENTATIVE AUTORADIOGRAPHS OF MID BRAIN SECTIONS SHOWING LOCAL CEREBRAL GLUCOSE UTILIZATION DURING THE SEIZURE PHASE. RATS WERE TREATED AS DESCRIBED IN THE EXPERIMENTAL DESIGN SECTION; ATROPINE WAS THE PRETREATMENT AGENT. EACH BAR REPRESENTS LOCAL CEREBRAL GLUCOSE UTILIZATION OF THE INDICATED STRUCTURE EXPRESSED AS PERCENT OF VEHICLE CONTROL FOR THE DESIGNATED TREATMENT.

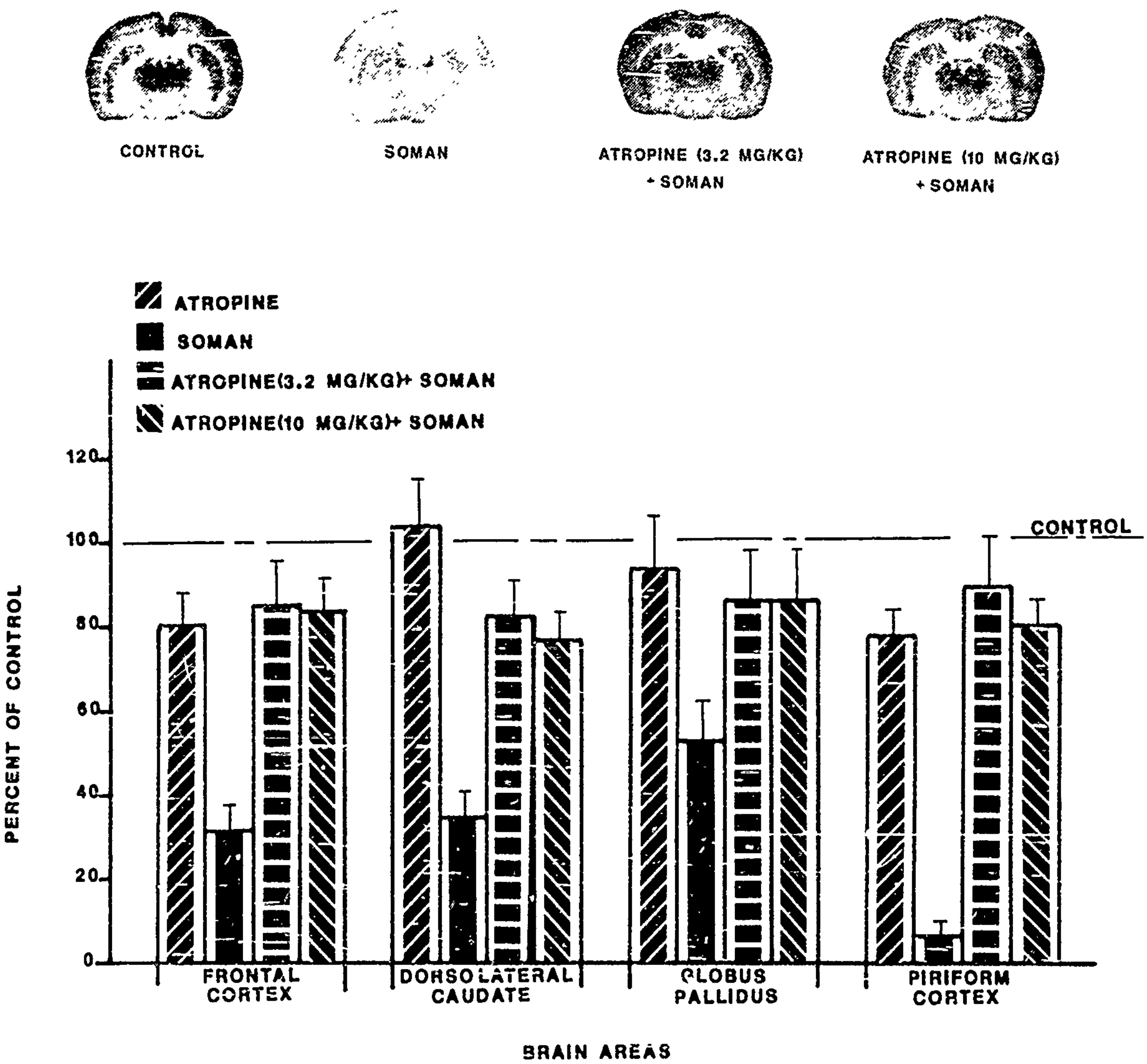


FIGURE 2A. REPRESENTATIVE AUTORADIOGRAPHS OF FRONTAL BRAIN SECTIONS SHOWING LOCAL CEREBRAL GLUCOSE UTILIZATION DURING THE PATHOLOGY PHASE. RATS WERE TREATED AS DESCRIBED IN THE EXPERIMENTAL DESIGN SECTION; ATROPINE WAS THE PRETREATMENT AGENT. EACH BAR REPRESENTS LOCAL CEREBRAL GLUCOSE UTILIZATION OF THE INDICATED STRUCTURE EXPRESSED AS PERCENT OF VEHICLE CONTROL FOR THE DESIGNATED TREATMENT.

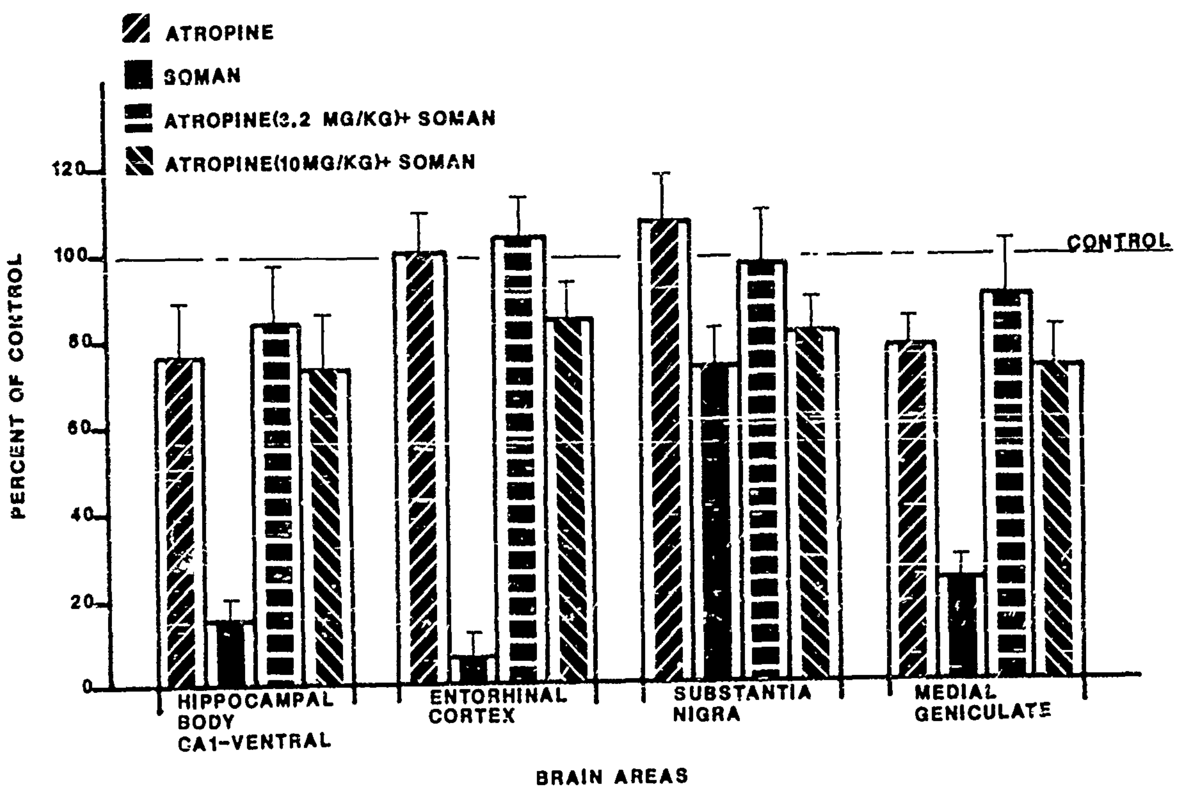
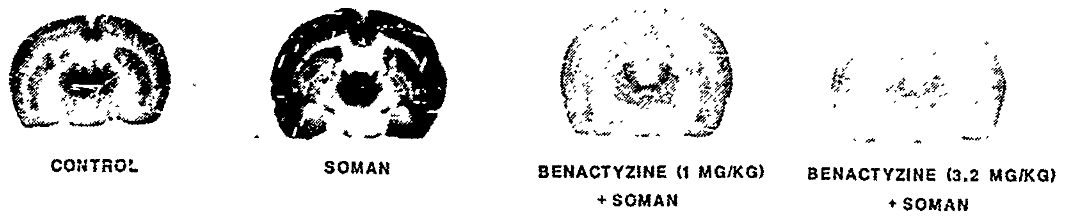


FIGURE 2B. REPRESENTATIVE AUTORADIOGRAPHS OF MID BRAIN SECTIONS SHOWING LOCAL CEREBRAL GLUCOSE UTILIZATION DURING THE PATHOLOGY PHASE. RATS WERE TREATED AS DESCRIBED IN THE EXPERIMENTAL DESIGN SECTION; ATROPINE WAS THE PRETREATMENT AGENT. EACH BAR REPRESENTS LOCAL CEREBRAL GLUCOSE UTILIZATION OF THE INDICATED STRUCTURE EXPRESSED AS PERCENT OF VEHICLE CONTROL FOR THE DESIGNATED TREATMENT.



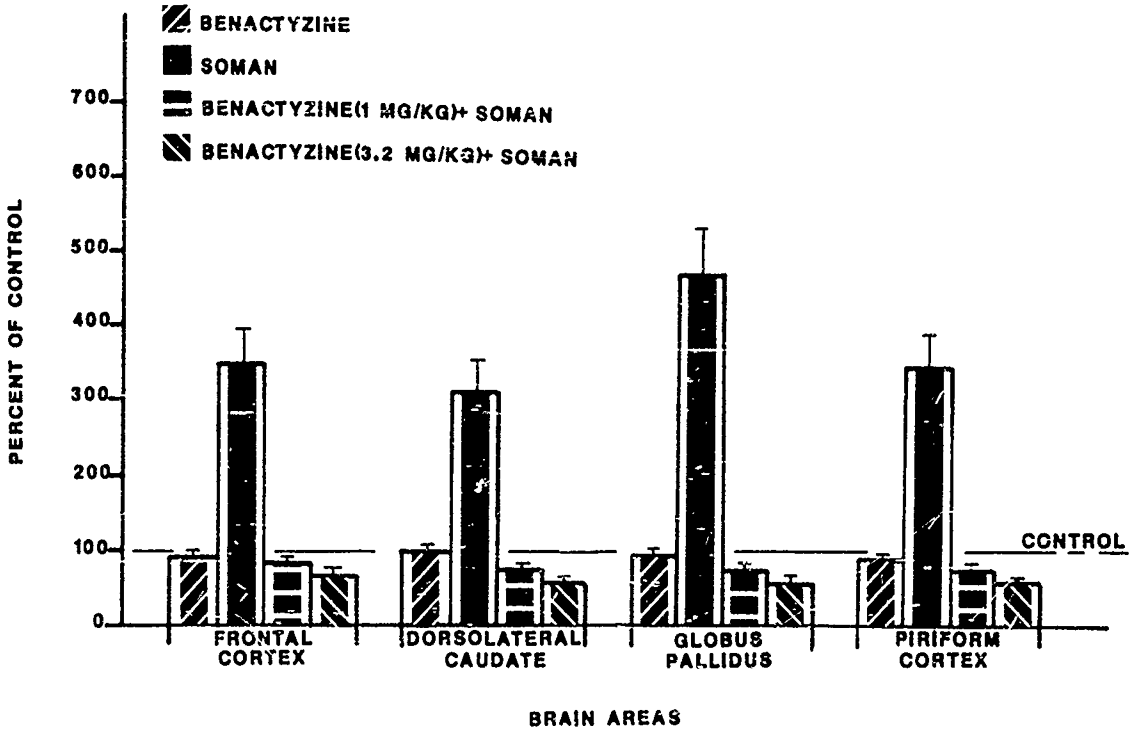


FIGURE 3A. REPRESENTATIVE AUTORADIOGRAPHS OF FRONTAL BRAIN SECTIONS SHOWING LOCAL CEREBRAL GLUCOSE UTILIZATION DURING THE SEIZURE PHASE. RATS WERE TREATED AS DESCRIBED IN THE EXPERIMENTAL DESIGN SECTION; BENACTYZINE WAS THE PRETREATMENT AGENT. EACH BAR REPRESENTS LOCAL CEREBRAL GLUCOSE UTILIZATION OF THE INDICATED STRUCTURE EXPRESSED AS PERCENT OF VEHICLE CONTROL FOR THE DESIGNATED TREATMENT.

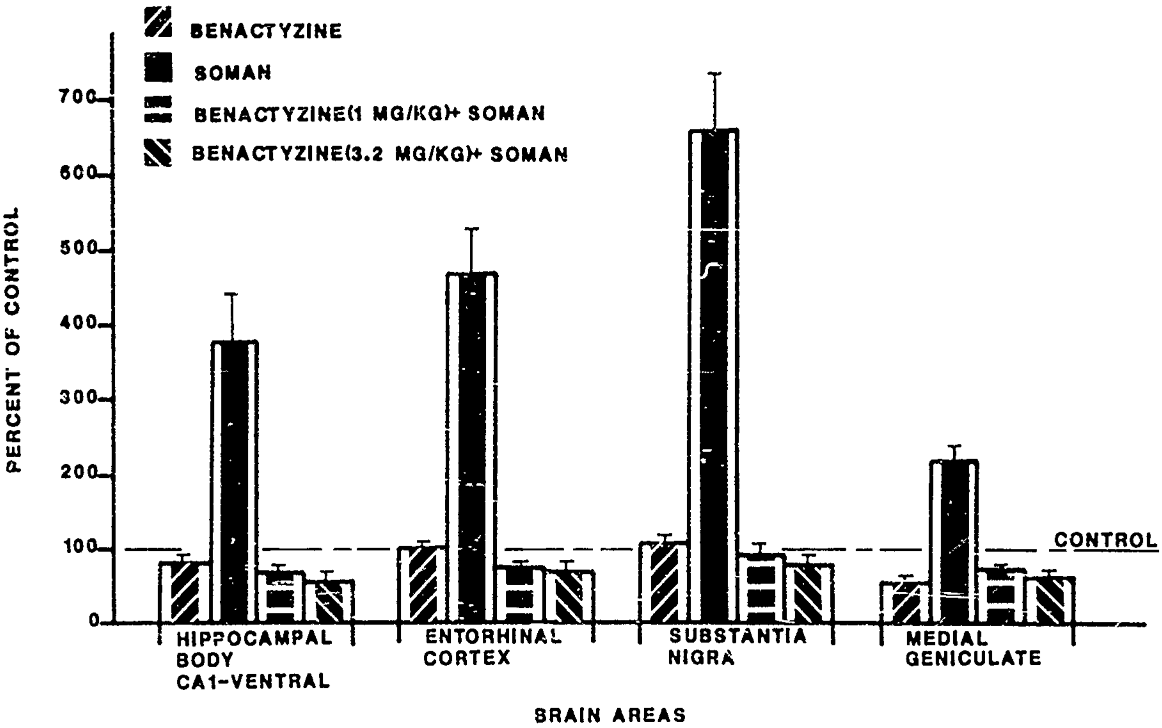


FIGURE 3B. REPRESENTATIVE AUTORADIOGRAPHS OF MID BRAIN SECTIONS SHOWING LOCAL CEREBRAL GLUCOSE UTILIZATION DURING THE SEIZURE PHASE. RATS WERE TREATED AS DESCRIBED IN THE EXPERIMENTAL DESIGN SECTION; BENACTYZINE WAS THE PRETREATMENT AGENT. EACH BAR REPRESENTS LOCAL CEREBRAL GLUCOSE UTILIZATION OF THE INDICATED STRUCTURE EXPRESSED AS PERCENT OF VEHICLE CONTROL FOR THE DESIGNATED TREATMENT.

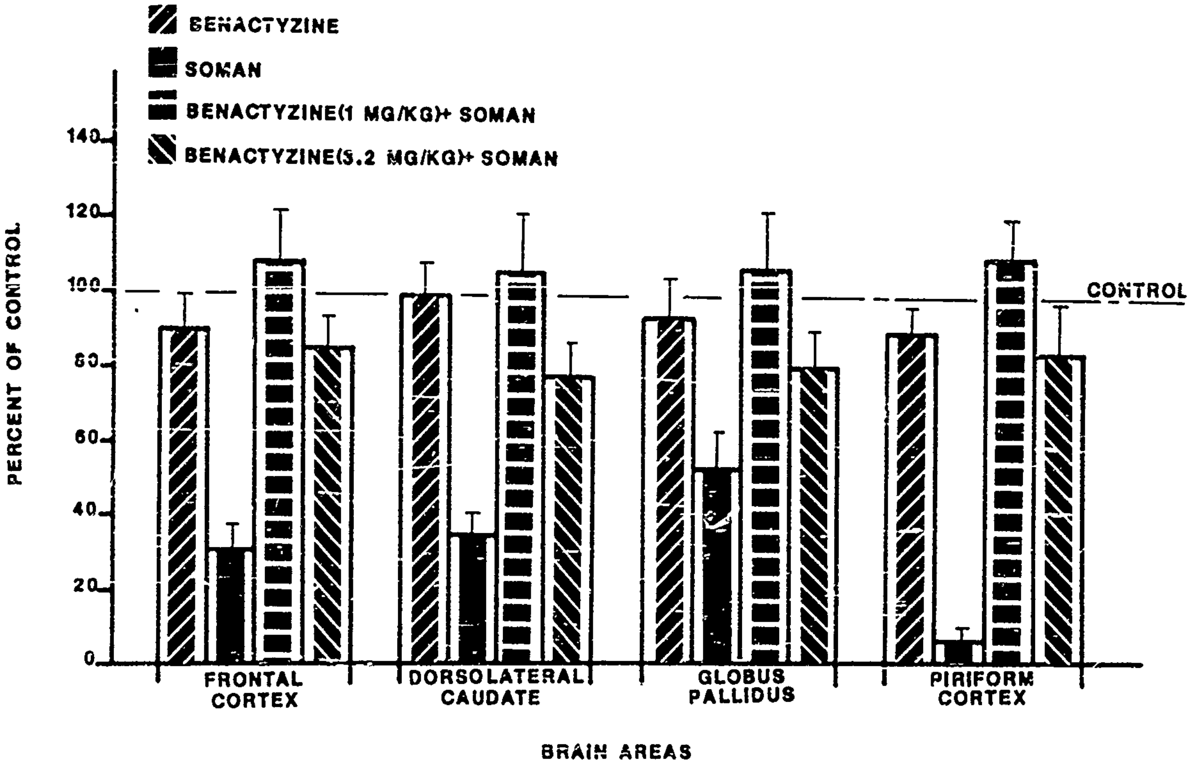


FIGURE 4A. REPRESENTATIVE AUTORADIOGRAPHS OF FRONTAL BRAIN SECTIONS SHOWING LOCAL CEREBRAL GLUCOSE UTILIZATION DURING THE PATHOLOGY PHASE. RATS WERE TREATED AS DESCRIBED IN THE EXPERIMENTAL DESIGN SECTION; BENACTYZINE WAS THE PRETREATMENT AGENT. EACH BAR REPRESENTS LOCAL CEREBRAL GLUCOSE UTILIZATION OF THE INDICATED STRUCTURE EXPRESSED AS PERCENT OF VEHICLE CONTROL FOR THE DESIGNATED TREATMENT.

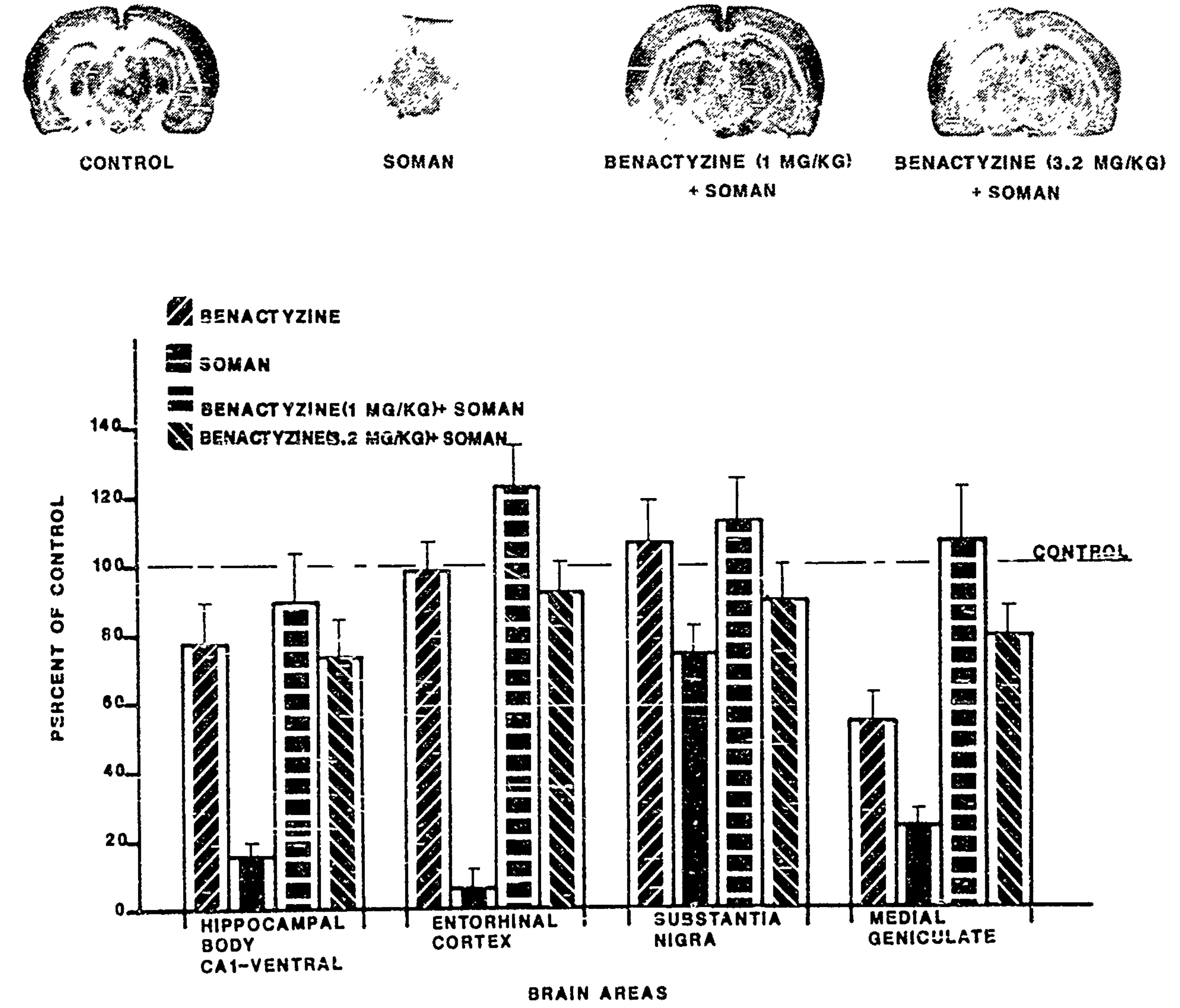


FIGURE 4B. REPRESENTATIVE AUTORADIOGRAPHS OF MID BRAIN SECTIONS SHOWING LOCAL CEREBRAL GLUCOSE UTILIZATION DURING THE PATHOLOGY PHASE. RATS WERE TREATED AS DESCRIBED IN THE EXPERIMENTAL DESIGN SECTION; BENACTYZINE WAS THE PRETREATMENT AGENT. EACH BAR REPRESENTS LOCAL CEREBRAL GLUCOSE UTILIZATION OF THE INDICATED STRUCTURE EXPRESSED AS PERCENT OF VEHICLE CONTROL FOR THE DESIGNATED TREATMENT.

SUMMARY

A. SOMAN: A SINGLE NEAR-LETHAL DOSE OF SOMAN PRODUCED REPETITIVE CONVULSIONS AND EXTENSIVE PERIPHERAL CHOLINOMIMETIC EFFECTS. CONSPICUOUS BRAIN DAMAGE WAS PRESENT IN THE BRAINS OF THESE RATS 72 HR POST SOMAN EXPOSURE. DAMAGE WAS MOST EXTENSIVE IN THE PIRIFORM/ENTORHINAL CORTEX AND AMYGDALA. LCGU WAS MARKEDLY INCREASED DURING THE SEIZURE PHASE (15 MIN POST SOMAN) AND DECREASED DURING THE PATHOLOGY PHASE (72 HR POST SOMAN).

B. ATROPINE + SOMAN: ATROPINE PRETREATMENT (3.2 OR 10 MG/KG) FAILED TO ABOLISH CONVULSIONS INDUCED BY SOMAN, ALTHOUGH THE DURATION OF CONVULSIONS WAS REDUCED, ESPECIALLY WITH THE HIGH DOSE. PERIPHERAL CHOLINOMIMETIC EFFECTS WERE SUBSTANTIALLY REDUCED. DURING THE SEIZURE PHASE, THE LCGU PATTERN WITH ATROPINE (3.2 MG/KG) WAS SIMILAR TO THAT WITH SOMAN ALONE, WHEREAS, THE LCGU PATTERN WITH ATROPINE (10 MG/KG) WAS SIMILAR TO CONTROLS. DURING THE PATHOLOGY PHASE, LCGU VALUES WERE SIMILAR TO CONTROL VALUES WITH BOTH DOSES OF ATROPINE AND CONSPICUOUS PATHOLOGY WAS NOT PRESENT.

C. BENACTYZINE + SOMAN: BENACTYZINE PRETREATMENT (1.0 OR 3.2 MG/KG) PROVIDED SUBSTANTIAL PROTECTION AGAINST SOMAN-INDUCED SEIZURES AND PERIPHERAL CHOLINOMIMETIC EFFECTS. LCGU 15 MIN POST SOMAN EXPOSURE WAS NEAR NORMAL WITH THE LOW BENACTYZINE DOSE AND GENERALLY SUPPRESSED IN MOST BRAIN STRUCTURES WITH THE HIGH DOSE. DURING THE PATHOLOGY PHASE, LCGU VALUES WERE WITHIN THE CONTROL RANGE AND CONSPICUOUS PATHOLOGY WAS NOT PRESENT.

CONCLUSIONS

A. SOMAN AT NEAR-LETHAL DOSES PRODUCES PROLONGED SEIZURE ACTIVITY THAT LEADS TO CONSPICUOUS BRAIN DAMAGE.

B. REDUCTION OF EITHER THE INTENSITY OR DURATION OF SEIZURE ACTIVITY WILL PROVIDE PROTECTION AGAINST SEIZURE-INDUCED BRAIN DAMAGE.

C. THE LOW DOSE OF ATROPINE (3.2 MG/KG) DID NOT REDUCE THE INTENSITY OF SEIZURES AS ASSESSED BY LCGU, BUT DID REDUCE THE DURATION OF SEIZURE ACTIVITY.

D. WITH THE HIGH DOSE OF ATROPINE (10 MG/KG) AND ESPECIALLY THE HIGH DOSE OF BENACTYZINE (3.2 MG/KG), LCGU WAS ACTUALLY SUPPRESSED IN MANY BRAIN REGIONS 15 MIN POST SOMAN EXPOSURE. THUS, SOMAN MAY HAVE BOTH EXCITATORY AND INHIBITORY ACTIONS ON THE BRAIN. HIGH DOSES OF MUSCARINIC ANTAGONISTS APPEAR TO SELECTIVELY BLOCK THE EXCITATORY COMPONENTS OF SOMAN'S ACTIONS.

E. PRETREATMENT WITH BOTH DOSES OF ATROPINE AND BENACTYZINE PROVIDED PROTECTION AGAINST THE DEVASTATING EFFECTS OBSERVED IN THE BRAIN 72 HR POST SOMAN EXPOSURE. MARKED REDUCTIONS IN LCGU AND CONSPICUOUS PATHOLOGY OBSERVED 72 HR POST SOMAN EXPOSURE WERE NOT OBSERVED IN THE PRETREATED RATS.

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11. Performance

SYNERGISTIC EFFECTS OF EXERCISE, CHEMICAL WARFARE PRETREATMENT DRUGS, ANTIDOTES AND THERAPEUTIC DRUGS ON RHESUS MONKEYS PERFORMING A COGNITIVE TASK

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INTRODUCTION

Studies have shown that pretreatment and antidote drugs for chemical warfare defense can degrade performance especially in the absence of nerve agent challenge. The pretreatment drug, pyridostigmine bromide, can be taken at a dose of 30 mg every eight hours with little behavioral effect. Pralidoxime chloride, at clinical dosages, also appears to have minimal effects. Atropine sulfate, on the other hand, shows effects that are both dose related and task specific. A realistic chemical warfare scenario in Naval operations may include the combined use of both pretreatment and antidote compounds in addition to therapeutic drugs such as scopolamine hydrobromide or meclizine hydrochloride to counteract motion sickness. The purpose of this study was to determine the effect of these compounds on reaction time in an exercising monkey.

METHOD

Subjects Four male juvenile rhesus monkeys (*Macaca mulatta*) with a mean body mass of 3.2 kg were used.

Apparatus A Plexiglas and metal chair located in a sound isolated chamber provided a device for the monkey to exercise on (see Figure 1). Limb movements by the monkey, which simulated a rowing motion, were required to move hand and foot pedals against a 1 kg load. A green pilot lamp and response lever were mounted on the chair in front of the monkey for the reaction time response. A pellet feeder on the chamber delivered food pellets to the monkey.

Procedure The monkeys were trained to perform the rowing motion to turn on a green pilot lamp and then pull a response lever to receive a food pellet. The monkeys' performance of this task was sufficient to raise heart rates above 200 bpm. Each monkey was given three 15 min work periods daily, each followed by a 5 min rest period. Reaction time was measured from the moment that the green pilot lamp appeared, (presented on a random ratio schedule of exercise responses) until the response lever was pulled. Food pellets were delivered if reaction times were less than 1 sec. Once a stable exercise rate was achieved, drug testing began utilizing a repeated measures experimental design with order of drug testing randomized. The

pretreatment and therapeutic drugs were administered 30 min prior to antidote administration. The antidotes were administered, once per week 30 min prior to behavioral testing, at 1, 2,, or 3 times the human equivalent dose provided by the U.S. Army Mark IV autoinjectors. Drug dosages used are given in Table 1. Drugs were administered intramuscularly in equal volumes using saline as the vehicle. Meclizine hydrochloride was mixed with peanut butter and given orally. Sham treatments consisting of either saline injections or peanut butter without meclizine were also given.

TABLE 1

Antidotes	Autoinjector Equivalents		
	1	2	3
Atropine sulfate mg/kg	.03	.05	.09
Pralidoxime chloride mg/kg	8.6	15.3	27.2

Pretreatment

Pyridostigmine bromide .41 mg/kg

Therapeutic

Scopolamine hydrobromide 8 μg/kg

Meclizine hydrochloride .71 mg/kg

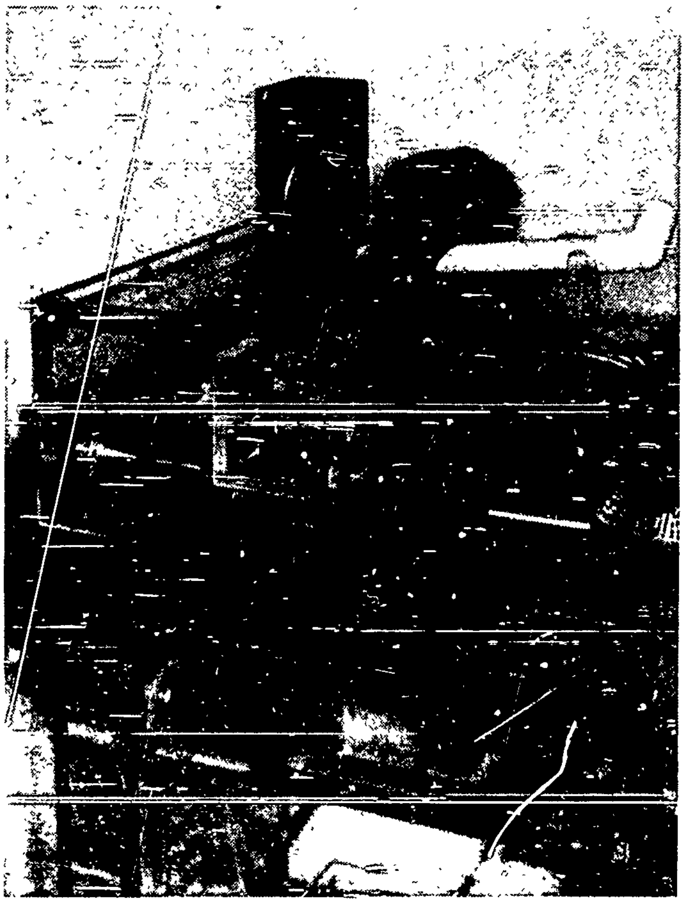

Figure 1. Plexiglas and metal exercise chair for the rhesus monkey.

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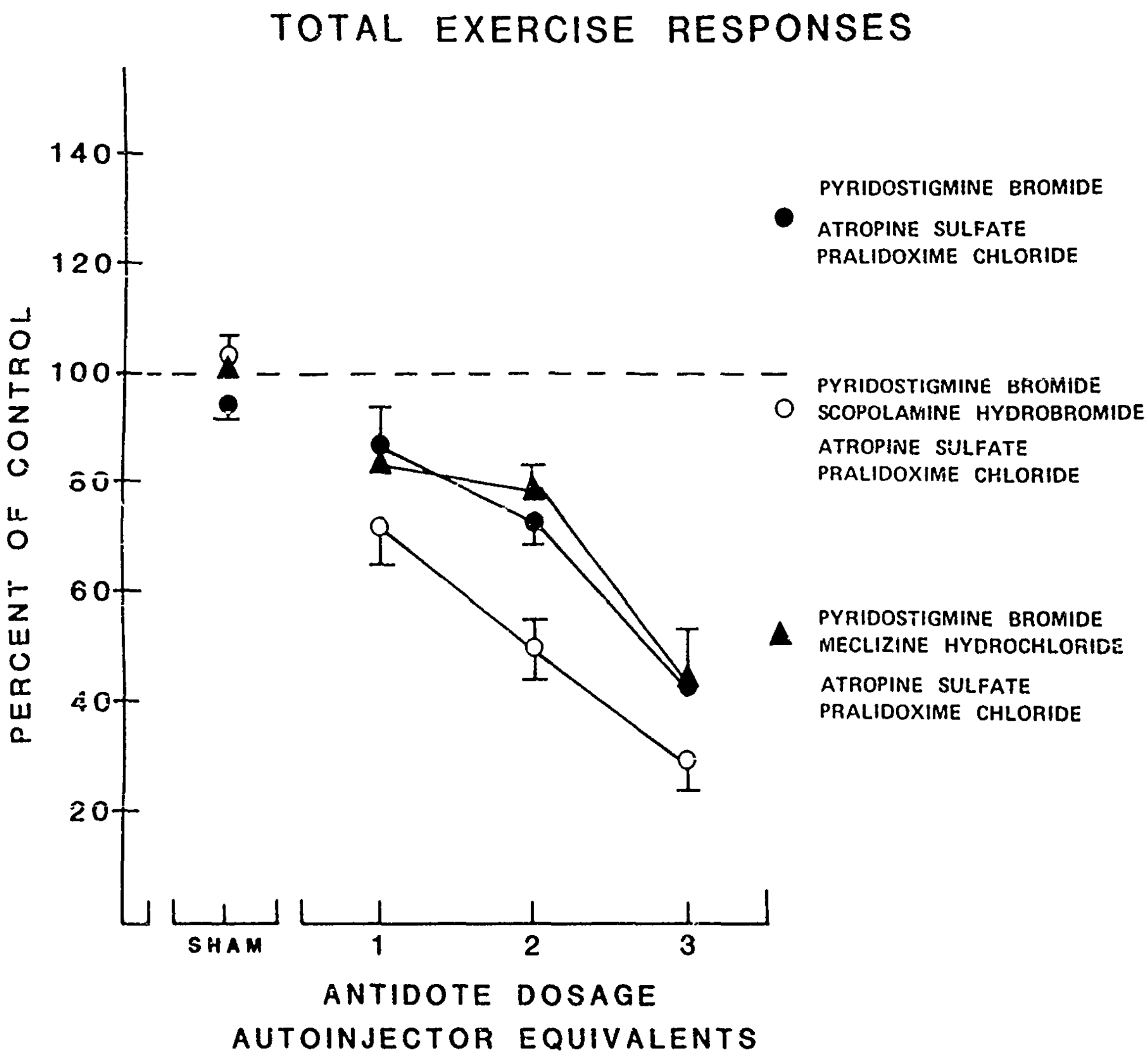


Figure 2. Mean exercise rate, as a percent of control rates, is shown for three antidote dosages with pyridostigmine, and also with the therapeutic drugs scopolamine and meclizine.

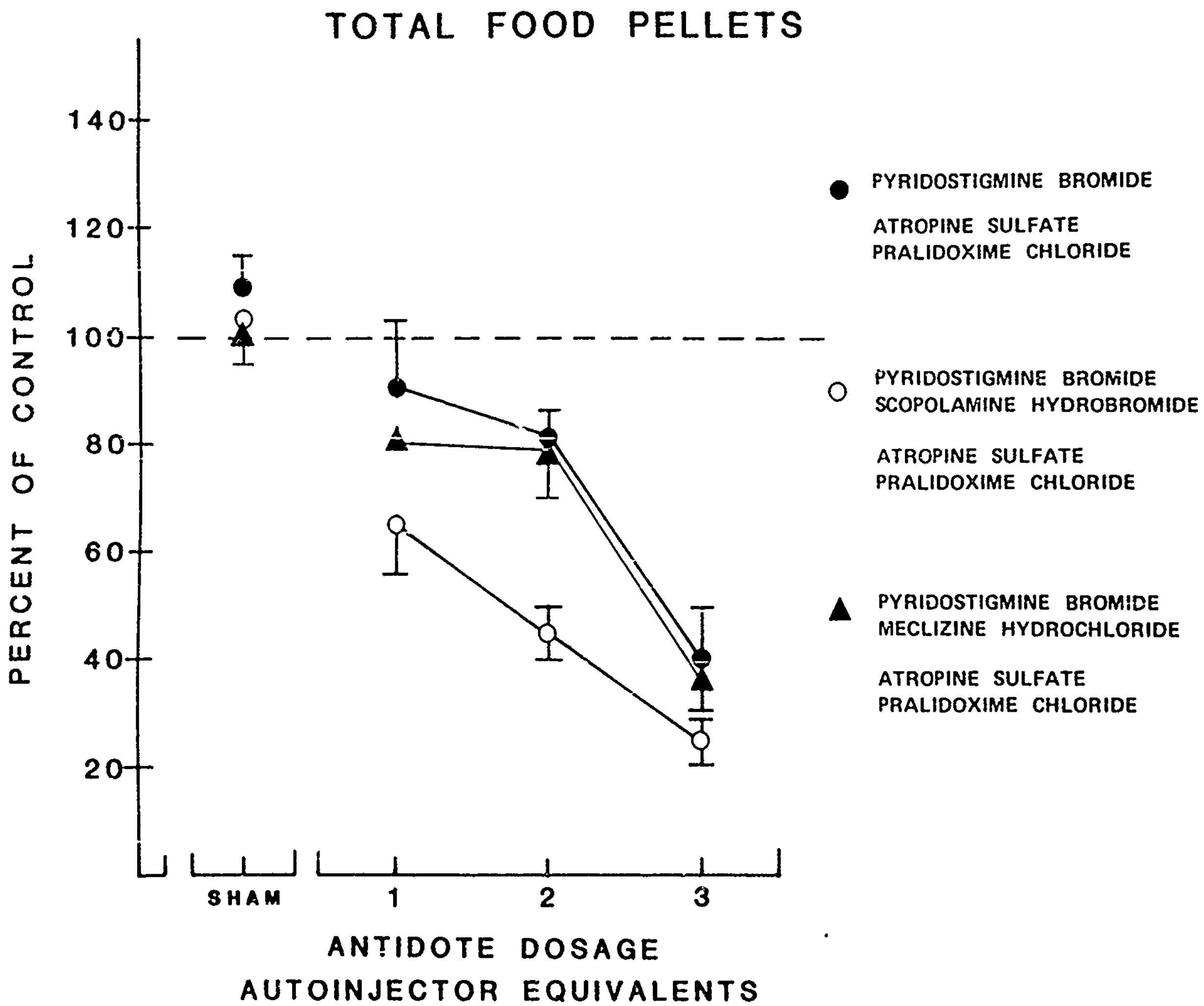


Figure 3. Mean food pellets, as a percent of control rates, is shown for three antidote dosages with pyridostigmine, and also with the therapeutic drugs scopolamine and meclizine.

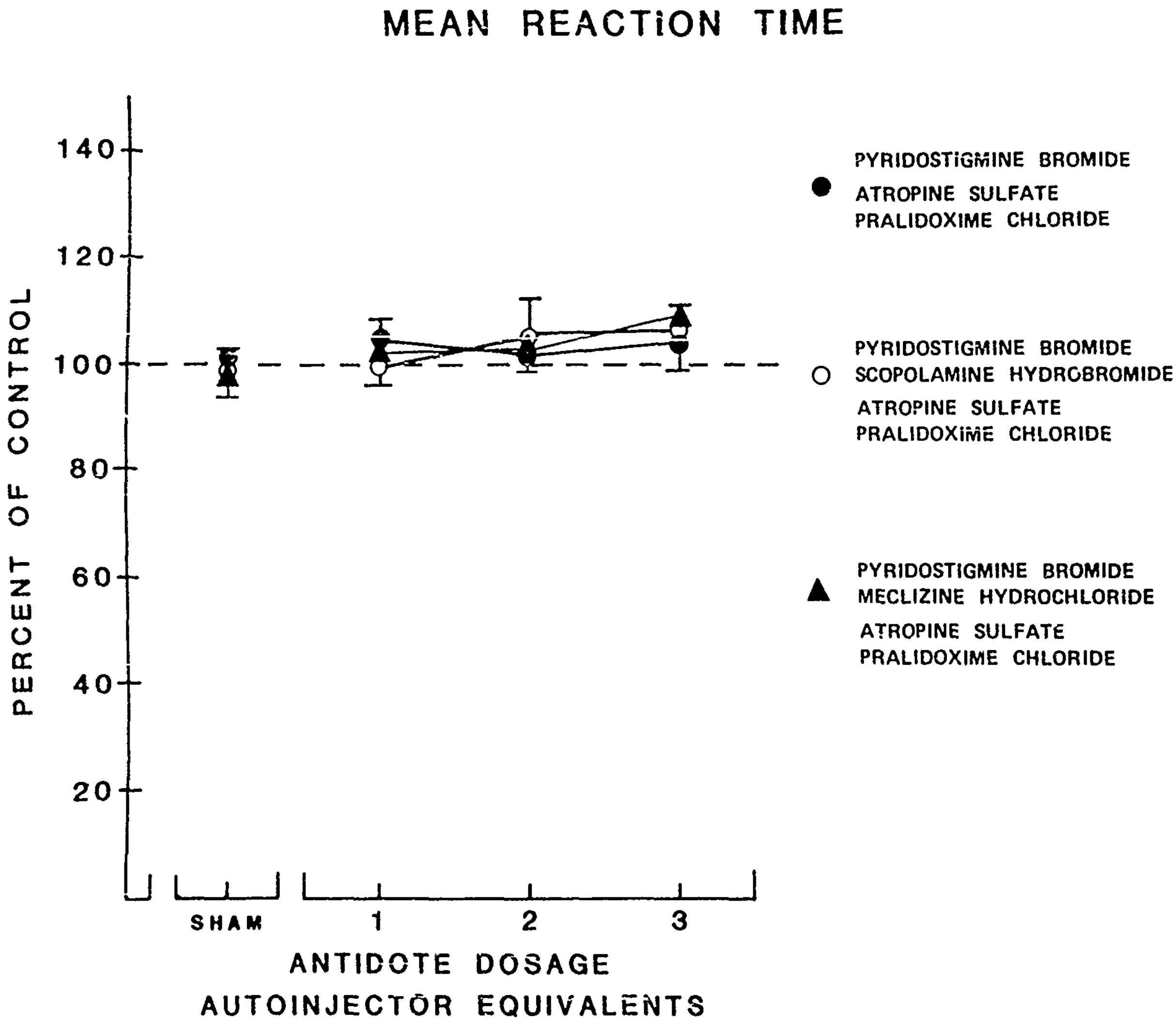


Figure 4. Mean reaction time, as a percent of control rates, is shown for three antidote dosages with pyridostigmine, and also for the therapetuic drugs scopolamine and meclizine.

RESULTS

The sham treatments did not alter exercise rate, food pellets earned, or reaction time when compared with control rates (preceeding session).

Analysis of variance was used to evaluate the effects of drug treatments. The mean exercise rate during drug testing is shown, as a percent of control rates, in Figure 2. The pretreatment drug pyridostigmine and the antidotes atropine and pralidoxime significantly reduced exercise rate below control values [$F\ (3, 9) = 23.3$, $P < .001$]. This reduction in response rate was negatively correlated with antidote dosage ($r = -.87$, $P < .01$). The action of meclizine to the drug combination did not alter this outcome ($P > .05$). The addition of scopolamine, however, did significantly reduce exercise rate below the effects produced by the pretreatment and antidote drugs alone [$F\ (1, 3)=31.8$, $P < .01$].

As expected because of reduced exercise rate, the pretreatment and antidote drugs also significantly reduced the number of food pellets earned [$F(3,9) = 21.5$, $P < .001$] as shown in Figure 3. The addition of meclizine had no significant effect on the dose response curve produced by the pretreatment and antidote drugs ($P > .05$). Scopolamine, however, significantly reduced the dose response curve produced by the pretreatment and antidote drugs alone [$F\ (1,3) = 40.2$, $P < .001$].

The mean reaction time to the food signals is shown, as a percent of control values, in Figure 4. The pretreatment and antidote drugs, at the dosages tested, had no significant effect of mean reaction time ($P > .05$). The therapeutic drugs meclizine and scopolamine, when added to the drug combination, did not alter this outcome.

CONCLUSIONS

Performance of a strenuous exercise task in the rhesus monkey is significantly reduced by the drug combination of the pretreatment drug pyridostigmine and the antidotes atropine and pralidoxime. The effect is dose related and is exaggerated by the antimotion sickness drug scopolamine but not meclizine. In contrast, the drug combinations tested had little effect on the performance of a simple reaction time task.

MEDICAL MATERIEL FOR CHEMICAL WARFARE DEFENSE

COL R.J. Summary, Chief, Field Materiel Development Division
US Army Medical Bioengineering Research and Development Laboratory
Fort Detrick, Frederick, Maryland 21701

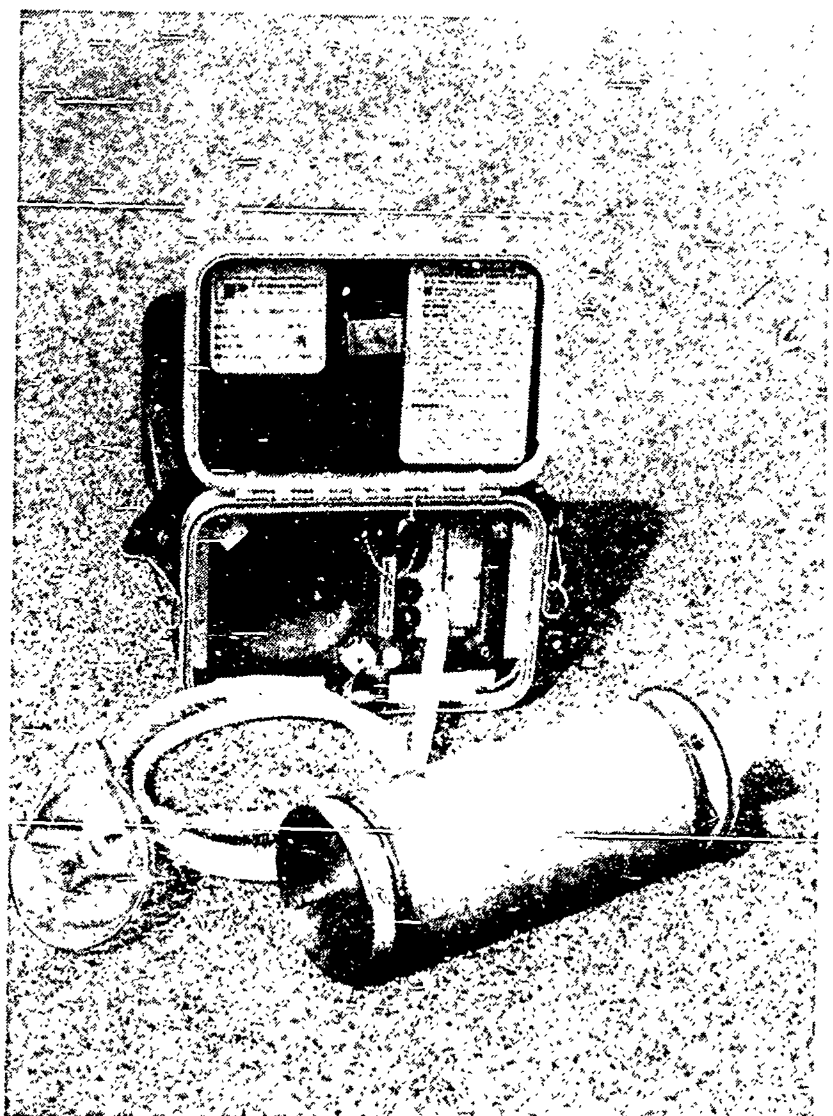
MISSION:

CONDUCTS ENGINEERING RESEARCH AND DEVELOPMENT OF MILITARY MEDICAL EQUIPMENT FOR THE ARMY AND ON AN AS-REQUIRED BASIS FOR THE NAVY AND AIR FORCE. CONSTRUCTS INITIAL PILOT PROTOTYPES, TEST MODELS, AND PRODUCES LIMITED QUANTITIES OF MEDICAL MATERIEL TO SUPPORT URGENT MILITARY REQUIREMENTS. CONDUCTS THE SURGEON GENERAL'S RESEARCH, DEVELOPMENT, TEST, AND EVALUATION (RDT&E) PROGRAM IN INTEGRATED VECTOR CONTROL SYSTEMS TO INCLUDE MATERIALS, METHODS, EQUIPMENT, AND CONCEPTS.

RESUSCITATOR / VENTILATOR, GAS-POWERED, INDIVIDUAL (GPV)

Objective: To develop a gas-powered resuscitator/ventilator for resuscitating chemical warfare agent casualties by field medical personnel either at Battalion Aid Stations, during evacuation, or at rear medical echelons.

Progress: Initial breadboard models manufactured by Puritan-Bennett Corp., Lexena, KS, and Mine Safety Appliances Research Center, Evans City, PA, have been subjected to Development Testing (DT) at the US Army Medical Bioengineering Research and Development Laboratory (USAMBRDL). The Puritan-Bennett model was found acceptable provided some minor redesign is accomplished. Efforts are currently under way to clarify requirements and prepare Joint Service Operational Requirement (JSOR) with the other services.

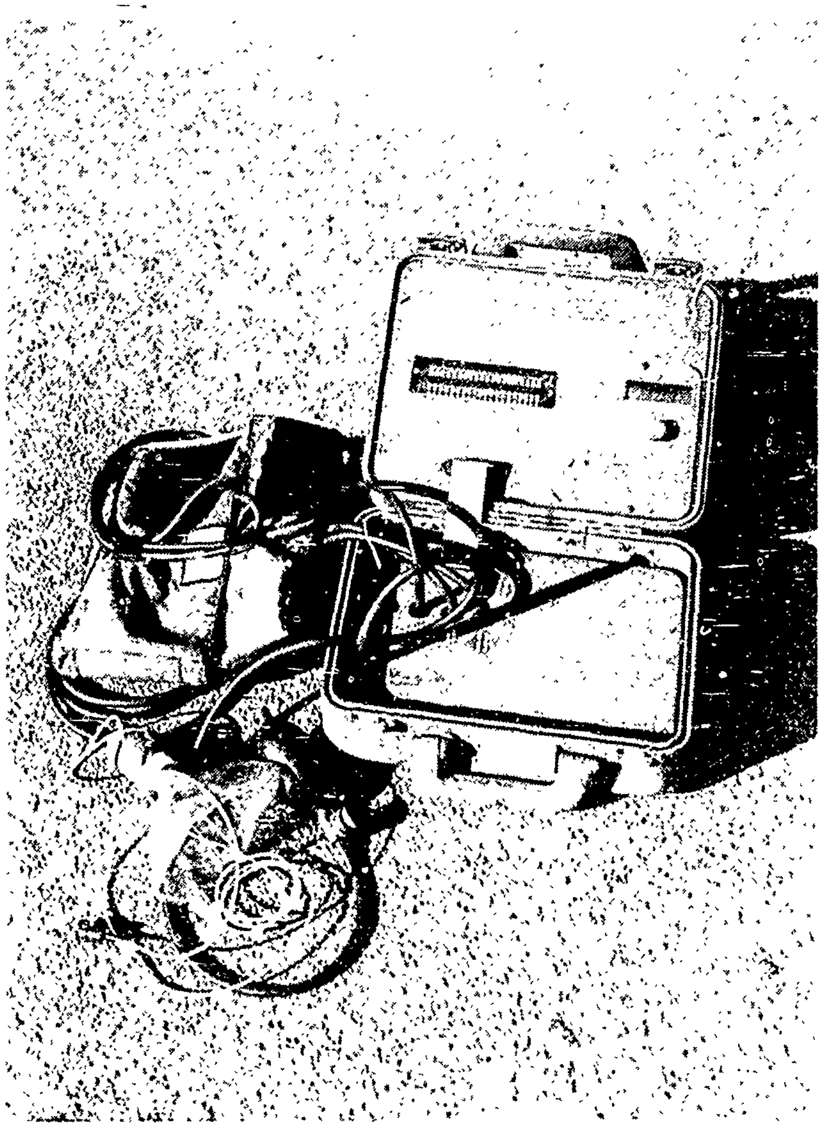


A-1282

NONINVASIVE NBC WARFARE PATIENT VITAL SIGNS MONITOR

Objective: To develop a device that will allow an aidman to detect noninvasively through chemically protective garments the vital signs (heart rate, blood pressure, respiration rate, and tidal volume) of an NBC casualty at field combat locations.

Progress: The GMS Engineering Corp., Columbia, MD, has designed and fabricated a breadboard instrument that has been successful in obtaining heart rate and blood pressure, especially in a moving M113 Armored Personnel Carrier (APC). Prototypes have been delivered, but significant improvements could be implemented by incorporating newer liquid crystal diodes (reduces power requirements) and by eliminating the hard copy printer. These design changes are currently being pursued.



CHEMICAL HARDENING OF FIELD LITTERS

Objective: To improve the existing litter so that it will not be degraded by chemical warfare agents, is easily decontaminated, and provides a surface on which casualties can be decontaminated.

Progress: Working in conjunction with the Chemical Research and Development Center (CRDC), Edgewood Area, MD, several samples of woven monofilament polypropylene fabrics with an open mesh weave were subjected to chemical warfare agent and decontaminating agent challenges. Preliminary results indicate that none of the fabrics are totally suitable; however, one fabric shows marked improvement over cotton duck. Development efforts are proceeding to locate other commercial fabrics, coatings which may be applied to the tested fabrics to improve chemical warfare agent resistance, or a supplemental disposable overwrap.

RESUSCITATION DEVICE, INDIVIDUAL, CHEMICAL

Objective: To develop a compact, manually operated device which can be operated by an individual soldier to ventilate chemical warfare agent casualties during initial frontline treatment.

Progress: Initial prototypes from Mine Safety Appliances Research Center, Evans City, PA, have been received and were subjected to in-house Development Testing (DT I). Prototypes were found to be acceptable, and the units are currently being evaluated for clinical acceptance. Field acceptance tests are scheduled to start during 4th Quarter FY85.

NBC CASUALITY NONINVASIVE (HEART RATE) SURVIVAL MONITOR

Objective: To develop a device that noninvasively determines the heart rate of a nuclear, biological, chemical (NBC) casualty's heart rate through chemically protective garments at field combat locations.

Progress: Three contractors have delivered breadboard models. These units have been found to work with varying degrees of sensitivity through chemical protective clothing, but they do not work satisfactorily in a moving M113 Armored Personnel Carrier (APC). These units have demonstrated that the imposed design requirements do not meet operational needs. Digital readout provides redundant treatment information and complicates design. Usage in a moving field ambulance introduces significant signal reduction challenges. These design requirements are currently being evaluated before proceeding with development.

CHEMICAL WARFARE AGENT PROTECTIVE PATIENT WRAP

Objective: To develop a field envelop to protect uncontaminated/decontaminated litter patients from chemical warfare agent exposure during movement through contaminated areas and to provide protection for litter patients awaiting evacuation or occupying cots/beds in treatment facilities when collective protection is not available.

Progress: In conjunction with the US Army Natick Research and Development Center (NRDC), Natick, MA, several protective wraps have been designed and fabricated. Selected fabric materials and closures are being tested at Dugway Proving Ground (DPG), UT. Respiratory testing is being conducted at CRDC, Edgewood Area, MD. Physiological testing will be conducted soon at the US Army Research Institute of Environmental Medicine (USARIEM), Natick, MA.

A-1287

PSYCHOACTIVITY OF ATROPINE IN NORMAL VOLUNTEERS

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ABSTRACT

THE SUBJECTIVE EFFECTS OF ATROPINE SULFATE INJECTIONS WERE ASSESSED IN NORMAL MALE VOLUNTEERS (N = 10), AS ONE PORTION OF A 3-PART STUDY (BEHAVIORAL, SUBJECTIVE AND PHYSIOLOGICAL EFFECTS OF ATROPINE). EACH VOLUNTEER WAS GIVEN 0, 2 OR 4 MG/70 KG ATROPINE SULFATE INTRAMUSCULARLY ACCORDING TO RANDOMIZED BLOCK SEQUENCES ON DIFFERENT TEST DAYS. TO ASSESS THE PSYCHOACTIVITY AND POSSIBLE ABUSE LIABILITY OF ATROPINE, TWO PREVIOUSLY VALIDATED PSYCHOMETRIC TESTS WERE GIVEN 1 HR BEFORE AND 1 HR FOLLOWING DRUG INJECTIONS. THESE TESTS WERE THE SINGLE DOSE QUESTIONNAIRE (SDQ) AND THE ADDICTION RESEARCH CENTER INVENTORY (ARCI) WHICH HAVE BEEN USED EXTENSIVELY TO EVALUATE THE SUBJECTIVE EFFECTS AND ABUSE LIABILITY OF A WIDE VARIETY OF CENTRALLY ACTIVE COMPOUNDS. DATA FROM THE SDQ INDICATED THAT ATROPINE PRODUCED SIGNIFICANT DISCRIMINATIVE EFFECTS, BUT DID NOT ELEVATE SCORES ON A DRUG-LIKING SCALE. DATA FROM THE ARCI INDICATED THAT ATROPINE PRODUCED SIGNIFICANT SEDATIVE-LIKE EFFECTS (PENTOBARBITAL-CHLORPROMAZINE-ALCOHOL SCALE), BUT DID NOT SIGNIFICANTLY ELEVATE SCORES ON THE EUPHORIA SCALE (MORPHINE-BENZEDRINE GROUP). THESE GROUP ANALYSIS, WHICH INDICATED LOW EUPHORIC EFFECTS OF ATROPINE, SUGGEST A LOW OVERALL ABUSE LIABILITY. HOWEVER, THE SIGNIFICANT SEDATIVE-LIKE PSYCHOACTIVE PROPERTIES OF ATROPINE IN CONJUNCTION WITH THE EUPHORIC EFFECTS IN 20% OF THE VOLUNTEERS SUGGEST THAT A SMALL, BUT SIGNIFICANT, NUMBER OF PERSONNEL MAY BE VULNERABLE TO ATROPINE ABUSE.

METHOD

SUBJECTS

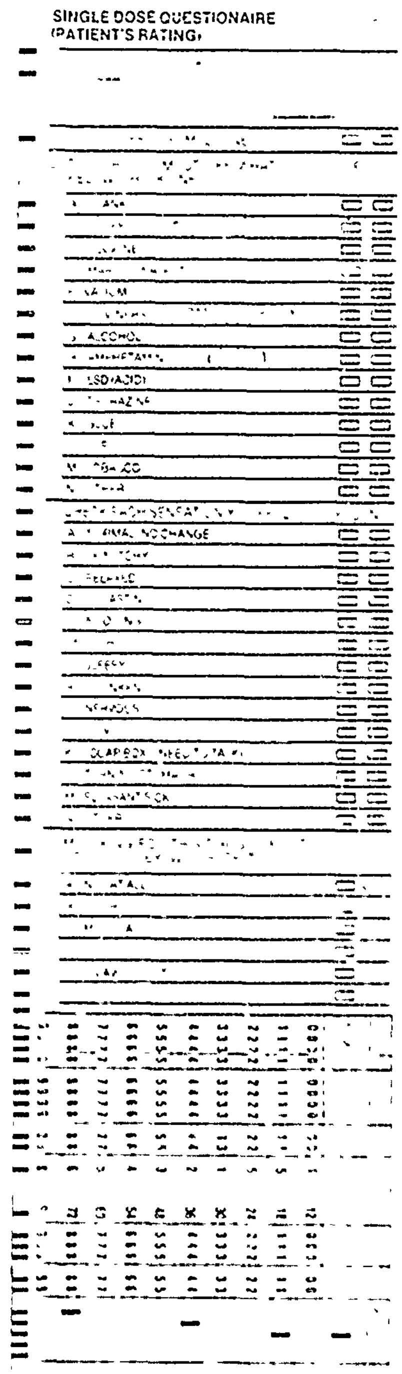
TEN MALE VOLUNTEERS WITH A MEAN AGE OF 26.1 YEARS (RANGE 22-31) PARTICIPATED IN THE STUDY. NONE OF THE PARTICIPANTS WAS A DRUG ABUSER OR HAD A HISTORY OF DRUG DEPENDENCE. EIGHT HAD LIMITED EXPERIENCE IN RECREATIONAL USE OF DRUGS OTHER THAN ALCOHOL. NONE OF THE PARTICIPANTS SMOKED CIGARETTES, AND ALL WERE PHYSICALLY FIT AND HEALTHY AT THE TIME OF THE STUDY. VOLUNTEERS WERE NOT PERMITTED TO DRINK ALCOHOL THE NIGHT BEFORE OR FOR TWO DAYS FOLLOWING AN EXPERIMENTAL SESSION AND WERE REQUIRED TO REMAIN FREE OF OTHER DRUGS FOR THE DURATION OF THE STUDY. BREATHALYZER AND PERIODIC URINALYSIS ASSURED COMPLIANCE. VOLUNTEERS WERE PARTICULARLY CAUTIONED NOT TO TAKE OVER-THE-COUNTER COLD MEDICATIONS DURING THE STUDY. ALL VOLUNTEERS WERE HIGH SCHOOL GRADUATES, FIVE HAD SOME COLLEGE, AND THRE WERE WORKING ON ADVANCED DEGREES. EACH VOLUNTEER GAVE HIS INFORMED WRITTEN CONSENT BEFORE PARTICIPATING IN THE STUDY.

PROCEDURE

QUESTIONNAIRES WERE ADMINISTERED TWICE ON EACH EXPERIMENTAL DAY APPROX IMATELY 1 HR BEFORE AND 1 HR AFTER DRUG INJECTION. THIRTY MINUTES AFTER AN INJECTION A SUBJECT WAS TESTED IN A COMPUTERIZED PURSUIT TRACKING TASK WHICH REQUIRED ABOUT 20 MIN OF VIGILANT PERFORMANCE. AFTER THE PURSUIT TRACKING TASK, A NUMBER OF VISUAL FUNCTIONS WERE EVALUATED BY AN OPTOMETRIST AND ROUTINE PHYSIOLOGIC OBSERVATIONS (HEART RATE, BLOOD PRESSURE, ETC) WERE MADE. VOLUNTEERS THEN FILLED OUT THE SDQ AND THE ARC WHICH REQUIRED ABOUT 5 MIN EACH TIME.

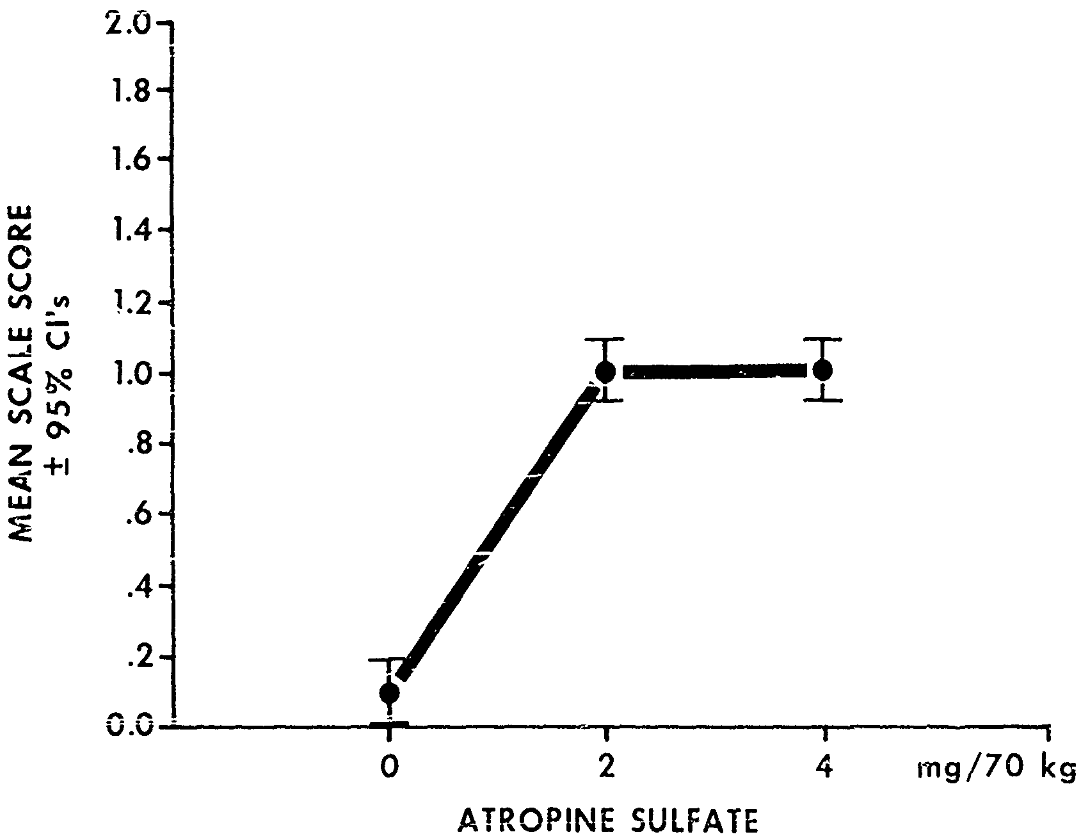
[1]Human subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to AR 70-25 and USAMRDC Reg 70-25 on the use of volunteers in research. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense (AR 360-5).

SINGLE DOSE QUESTIONNAIRE


SINGLE DOSE QUESTIONAIRE
(PATIENT'S RATING)

ALCOHOL
LSD (ACID)
NORMAL NO CHANGE

SDQ: PART I
FEEL DRUG?



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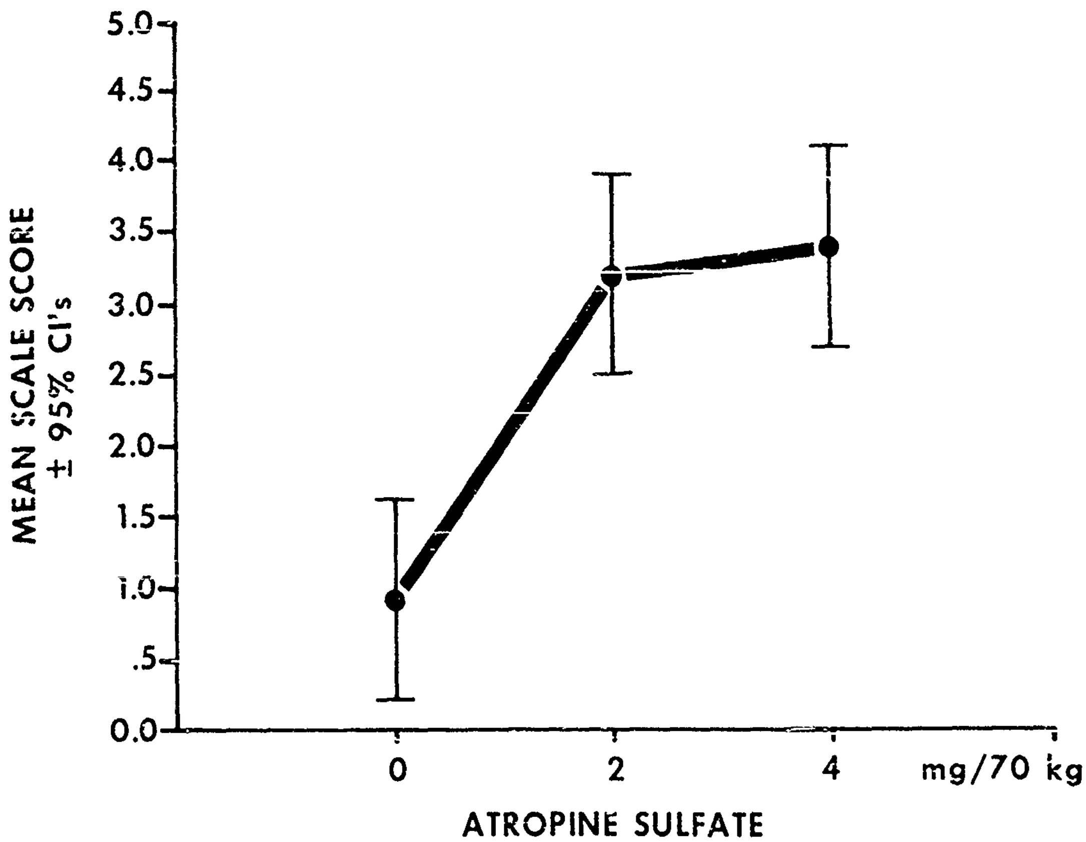
SDQ: PART III
SENSATIONS



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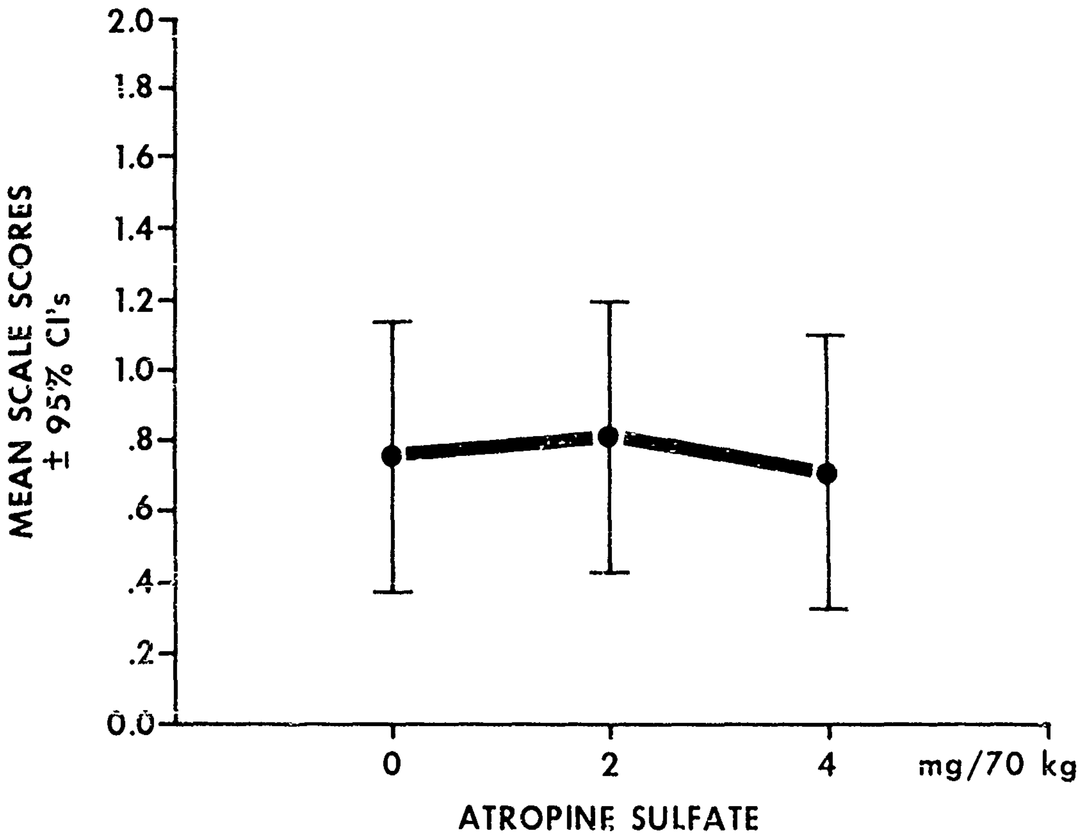
SDQ: PART IV
SUBJECT LIKING



ADDICTION RESEARCH CENTER INVENTORY

QUESTIONNAIRE

- [illegible]
- HAVE A FEELING OF JUST DRAGGING ALONG RATHER THAN COASTING
- FEEL AS IF I WOULD BE MORE POPULAR WITH PEOPLE TODAY
- TODAY I SAY THINGS IN THE EASIEST POSSIBLE WAY
- FEEL ANXIOUS AND UPSET
- FEEL MORE CLEAR HEADED THAN DREAMY
- [illegible]

QUESTIONNAIRE ARC [illegible]

INSTRUCTIONS: MARK [illegible] YES OR NO

21 MY HEAD FEELS HEAVY

22 I FEEL [illegible]PATIENT

23 I HAVE UNUSUAL WEAKNESS OF MY MUSCLES

24 I CAN COMPLETELY APPRE[illegible] WHAT OTHERS ARE SAYING [illegible] IN THIS MOOD

25 I FEEL LIKE AVOIDING [illegible] ALTHOUGH I USUALLY [illegible] FEEL THIS WAY

26 I WOULD BE HAPPY ALL THE TIME IF I FELT AS I FEEL NOW

27 I FEEL DIZZY

28 [illegible] ENERGY

29 I AM IN THE MOOD TO TALK ABOUT THE FEEL[illegible]

30 [illegible]

31 PEOPLE MIGHT SAY THAT I [illegible] A LITTLE DULL TODAY

32 IT SEEMS I'M SPENDING [illegible] THAN I SHOULD [illegible]

33 IT SEEMS HARDER THAN USUAL TO MOVE AROUND

34 I FEEL SO GOOD THAT [illegible] OTHER PEOPLE CAN TELL

35 MY HANDS FEEL [illegible]

36 [illegible] WHEN I TRY TO WRITE

37 I AM MOODY

38 I FEEL AS IF SOMETHING PLEASANT HAD [illegible] HAPPENED TO ME

39 I FEEL DROWSY

40 I FEEL MORE [illegible] THAN DREAMY

OBS. HOUR DAY YEAR FORM NO.

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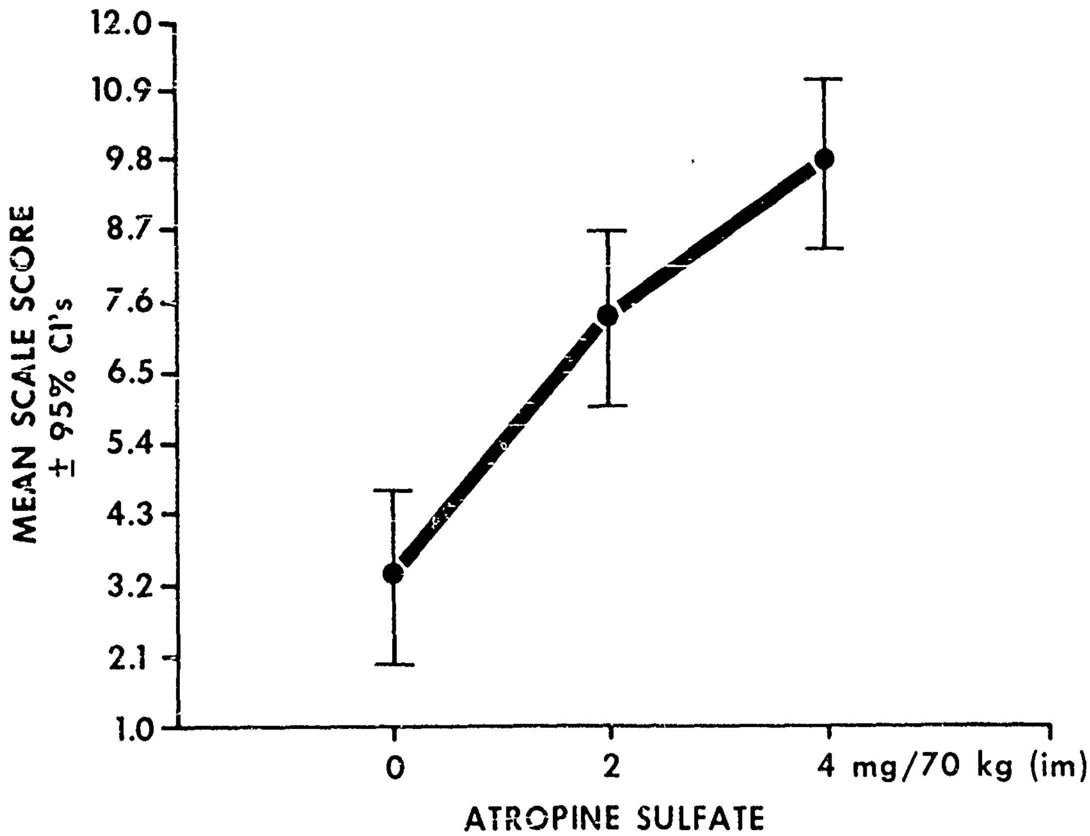
PENTOBARBITAL - CHLORPROMAZINE - ALCOHOL GROUP

(SEDATIVE/INTOXICATION)



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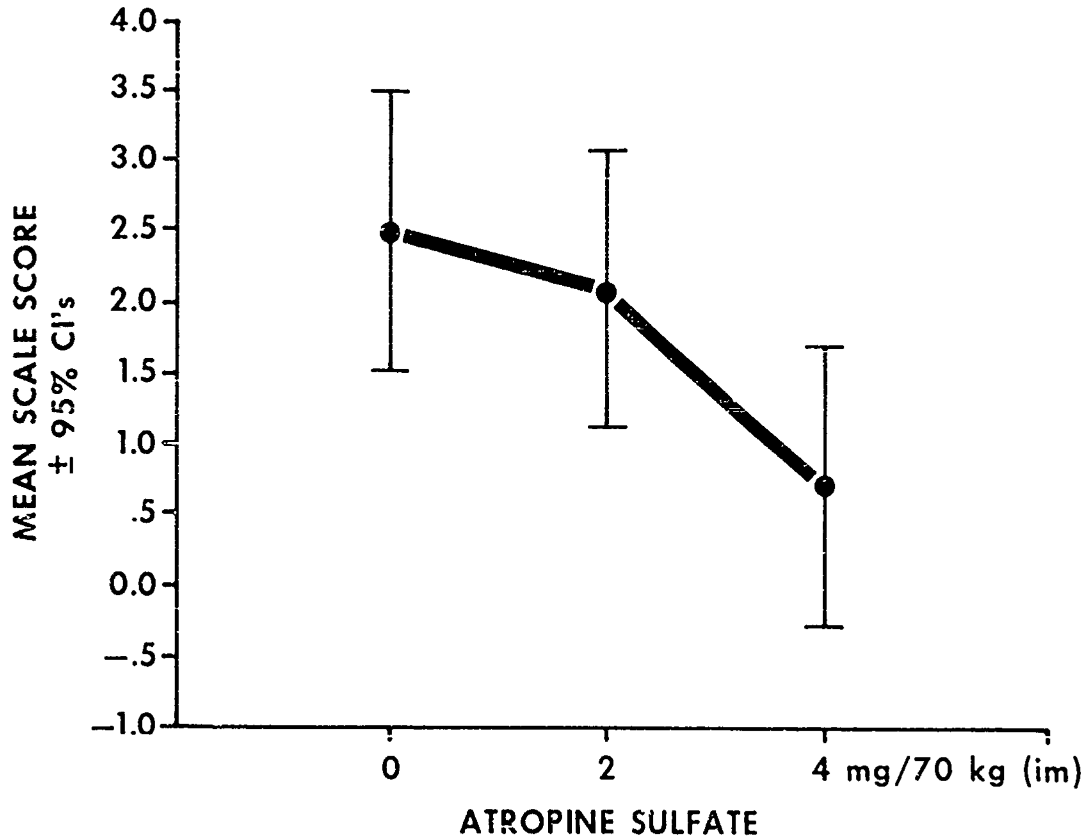
MORPHINE - BENZEDRINE GROUP (EUPHORIA)



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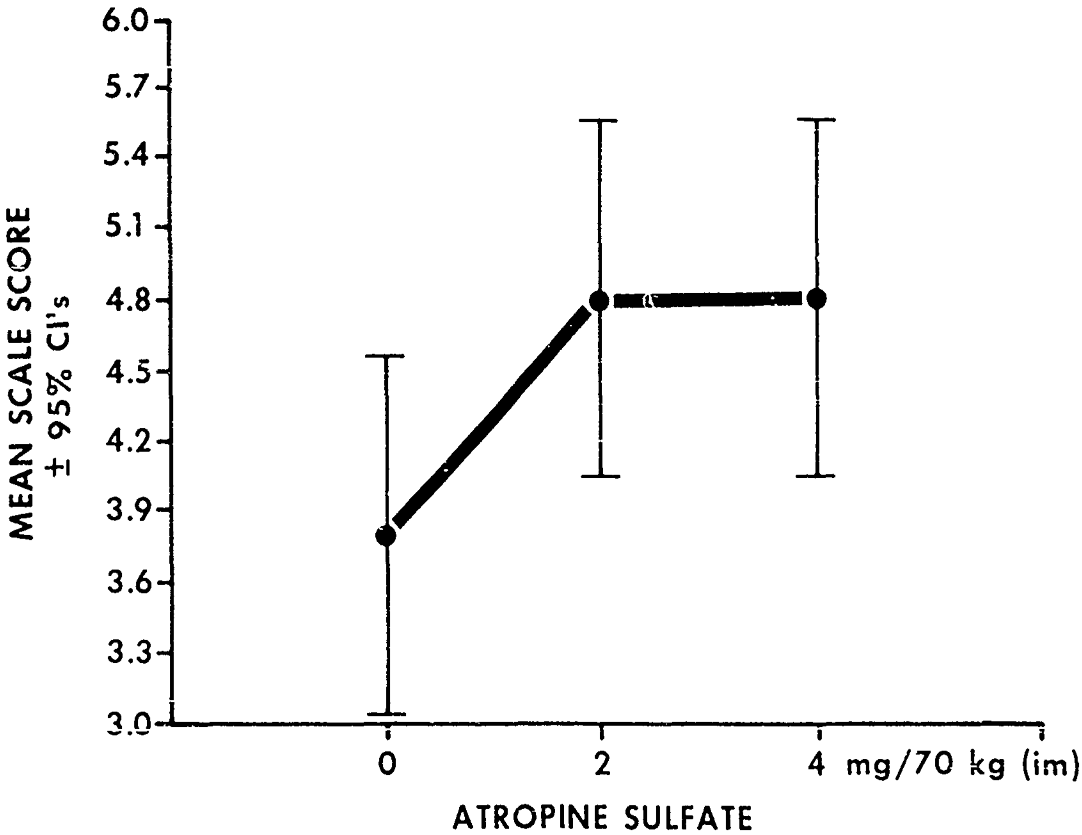
LSD SCALE (HALLUCINOGEN/DYSPHORIA)



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DISCUSSION

DATA FROM THE SINGLE DOSE QUESTIONNAIRE (SDQ) INDICATED THAT BOTH DOSES OF ATROPINE WERE PSYCHOACTIVE AND PRODUCED RELIABLY DISCRIMINATED SYMPTOMATIC EFFECTS. THE PSYCHOPHARMACOLOGIC PROFILE OF INTEROCEPTIVE EFFECTS PRODUCED BY ATROPINE WAS PERDOMINANTLY SEDATIVE-LIKE. SCORES ON EITHER, OR BOTH, THE EUPHORIANT SCALES (LIKING SCALE OF THE SDQ AND MBG SCALE OF THE ARCI) WERE ELEVATED IN CERTAIN INDIVIDUALS BUT NOT IN THE GROUP RESULTS. PREVIOUS RESEARCH HAS SHOWN THAT SCORES FROM THE MBG SCALE GENERALLY COVARY WITH "LIKING" SCALE SCORES, SUPPORTING THE NOTION THAT THIS SCALE REFLECTS EUPHORIC DRUG EFFECTS [5]. DOSE-RELATED INCREASES IN "LIKING" AND MBG SCALE SCORES ARE THE HALLMARK SUBJECTIVE EFFECTS OF ABUSED DRUGS AND DEFINE A DRUG AS A EUPHORIANT. ATROPINE DID NOT PRODUCE THE STRIKING DOSE-RELATED INCREASES IN SCORES ON THE EUPHORIA SCALES THAT ARE PRODUCED BY MOST DEPENDENCE-PRODUCING DRUGS IN KNOWN DRUG ABUSERS OR OF PSYCHOMOTOR STIMULANTS IN NORMAL AND DRUG-ABUSING VOLUNTEERS [5]. THEREFORE, APPARENTLY, ATROPINE HAS LIMITED ABUSE POTENTIAL. HOWEVER, SEDATIVES, ALCOHOL, AND POSSIBLY OTHER DRUGS, MAY SELECTIVELY PRODUCE ELEVATIONS IN EUPHORIA SCALES AMONG PERSONS WITH HISTORIES OF SEDATIVE ABUSE OR ALCOHOLISM [1-4]. THESE OBSERVATIONS SUGGEST TWO PRACTICAL IMPLICATIONS. FIRST, INSOFAR AS ATROPINE PRODUCED PREDOMINANTLY SEDATIVE-LIKE INTEROCEPTIVE EFFECTS, PERSONS WITH HISTORIES OF SEDATIVE ABUSE OR ALCOHOL WHO RECEIVE ATROPINE FOR THERAPEUTIC REASONS MAY REQUIRE SPECIAL ATTENTION. SECOND, REGARDLESS OF THE UNDERLYING MECHANISM, 10 TO 20 % OF NORMAL NONDRUG-ABUSING PERSONS MAY BE VULNERABLE TO ABUSE OF ATROPINE.

REFERENCES

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2. HENNINGFIELD, J. E., R. D. CHAIT AND R. R. GRIFFITHS. CIGARETTE SMOKING AND SUBJECTIVE RESPONSE IN ALCOHOLICS: EFFECTS OF PENTOBARBITAL. CLIN PHARMACOL THER 33: 806-812, 1983.

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CENTRAL MUSCARINIC ACTIONS OF PHYSOSTIGMINE AND OXOTREMORINE ON AVOIDANCE BEHAVIOR OF SQUIRREL MONKEYS

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Precise delineation of receptors relevant to the pharmacology of nerve agents and their relative central nervous system (CNS) involvement is required for an appropriate response to their potential use in warfare. Behavioral effects of nerve agents may be the result of actions at various peripheral (PNS) or CNS receptor sites or may be the result of the combined actions of agent in the CNS and in the periphery. Moreover, multiple cholinergic and non-cholinergic receptors may be involved upon exposure to nerve agents. The experimental study of drugs which poorly penetrate the CNS has been useful in dissociating the mechanism of action of these compounds. For example, whereas physostigmine reversibly inhibits acetylcholinesterase both in the CNS and in the PNS, the quaternary analog, neostigmine, acts preferentially in the PNS. Differences in the behavioral effects of these compounds has led to the conclusion that the behavioral effects of physostigmine are mediated by CNS mechanisms. However, this conclusion conflicts with the prevalent observation that physostigmine and neostigmine produce comparable alterations in behavioral performances, indicating that PNS receptors may also be involved. Thus, the study of these drugs, given alone, cannot precisely describe sites or mechanisms of action; additional analytical techniques are required. Compounds of high affinity for muscarinic cholinergic receptors in the CNS or PNS are available for this purpose.

Comparative drug-interaction studies were carried out in the present study to assess the involvement of central muscarinic receptors in the behavioral effects of physostigmine. The relative ability of the selective muscarinic antagonists, atropine or methylatropine, to reverse the the effects of physostigmine or the specific muscarinic agonist oxotremorine was evaluated. Behavior of squirrel monkeys was studied under conditions which, as in combat, arranged for increasing noxious consequences to follow performance-decrements. Avoidance behavior was maintained under a schedule in which every leverpress postponed electric shock delivery for 20 sec; shock occurred every 5 sec in the absence of responding. Cumulative doses (0.003-0.3 mg/kg, i.m.) of physostigmine or oxotremorine produced dose-related decreases in response rates and increases in response durations and in rates of shock delivery. Similar effects occurred with neostigmine (0.003-0.3 mg/kg, i.m.) or oxotremorine-M (0.001-0.1 mg/kg, i.m.), quaternary derivatives which poorly penetrate into the CNS. The peripherally-acting muscarinic agonist bethanechol (0.03-2.0 mg/kg, i.m.) decreased responding only at doses which produced profound peripheral manifestations. Methylatropine (1 mg/kg, i.m.) completely prevented the behavioral effects of neostigmine, oxotremorine-M, and bethanechol and also antagonized the peripheral parasympathetic effects of these compounds (salivation, urination, and vomiting) without having any effects of its own indicating its high efficacy for muscarinic receptor blockade in the periphery. Although methylatropine prevented parasympathetic effects, it did not influence the behavioral effects of physostigmine or oxotremorine. Atropine (1 mg/kg, i.m.) prevented the peripheral manifestations as well as the behavioral effects of physostigmine and of oxotremorine even though atropine decreased avoidance responding when given alone.

The use of compounds which act selectively at specific receptors in the brain and in the periphery provided definitive information regarding the mode of action of several cholinergic agents on behavior. Comparison of effects of the drugs administered alone would have led to erroneous conclusions; i.e., that physostimine produces its behavioral effects primarily by virtue of its peripheral actions. Taken as a whole, the drug interaction experiments revealed that behavioral effects of physostigmine involve a CNS component which depends, perhaps in total, upon muscarinic cholinergic receptors.

INTRODUCTION

Behavioral effects of physostigmine may be the result of actions in the central nervous system (CNS) or in the periphery (PNS). Although the experimental study of drugs which poorly penetrate the CNS has been useful in dissociating the sites of action of physostigmine, comparison of the effects of these drugs when given alone can lead to erroneous conclusions. Drug-interaction experiments are one approach to this problem (cf. Vaillant, 1967). Compounds with high specificity for muscarinic cholinergic receptors in the CNS and PNS were used in the present study to assess the involvement of central muscarinic receptors in the behavioral effects of physostigmine.

Vaillant, G. E. A comparison of antagonists of physostigmine-induced suppression of behavior. J. Pharmacol. Exp. Therap. 157: 636-648, 1967.

METHOD

Behavior of adult male and female squirrel monkeys (Saimiri sciureus) was studied under schedules of electric shock postponement (avoidance). The monkeys were seated in Plexiglas chairs in which they faced a response lever and stimulus lights. Depression of the lever with a force exceeding 20 g was recorded as a response. Experiments were conducted within individual acoustic isolation chambers.

Under the avoidance schedule, every leverpress postponed shock (AC, 200 msec constant current pulse, delivered to a distal section of the tail) for 20 sec. In the absense of responding, shock occurred every 5 sec; shock during this period defined an escape failure, and this never occurred under non-drug conditions. The avoidance schedule was operative during six 10 min segments in the presence of white lights. These 10 min periods were preceded and seperated by 10 min timeout periods during which the lights were off, no shocks were scheduled and responding had no scheduled consequences.

Drugs were administered cumulatively by injection of a successively higher dose at the beginining of each timeout period. The cholinesterse inhibitor, phsysostigmine sulfate (Sigma Chemical Co.), its quaternary analog, neostigmine methyl sulfate (Sigma Chemical Co.), and the muscarinic agonist oxotremorine sesquifumarate (Aldrich Chemical Co.) were studied in doses from 0.003 to 0.3 mg/kg. Oxotremorine methyl bromide (oxotremorine-M), a quaternary derivative of oxotremorine (donated by B. Ringdahl), was given in doses from 0.001 to 0.1 mg/kg. The quaternary choline ester, bethanechol chloride (Sigma Chemical Co.), was administered in doses of 0.03 to 3 mg/kg. Atropine sulfate and atropine methyl nitrate (Sigma Chemical Co.) were studied alone (1 mg/kg) and in combination with the other compounds. During drug interaction experiments, atropine or methylatropine was given in conjunction with the first injection of the test drug. All drugs were dissolved in 0.9% saline and administered in the calf muscle in a volume of 1 ml/kg. Drug doses are expressed as the salt.

Experimental events were controlled and data were collected by a PDP 11/23 computer operating under SKED software. The following measures were recorded seperately for each 10 min segment of the session: response rate (responses per sec), response duration (sec per response) measured with a 5 msec time base, shock rate (shocks per sec), and number of escape failures. Each animal served as its own control.

RESULTS

Avoidance behavior was characterized by relatively constant rates of responding ranging from 0.29 to 1.77 responses per sec across animals. Response durations were relatively constant throughout the session and ranged, across animals, from 0.20 to 3.00 sec per response. Few if any shocks were delivered under control conditions.

Effects of oxotremorine or physostigmine alone or in combination with atropine are shown in FIG. 1. Oxotremorine produced small increases in response rate at lower doses and decreased responding at doses of 0.1 mg/kg and higher. Physostigmine decreased responding at 0.3 mg/kg. Response durations were increased by doses of oxotremorine or physostigmine which decreased rates of responding. Rates of shock presentation and instances of escape failures occurred at doses of these drugs which did not influence the rate or duration of response. Marked parasympathetic actions (salivation, urination, defecation, and sometimes emesis) of oxotremorine or physostigmine were noted at doses of 0.1 mg/kg and greater; all of these effects were completely prevented by atropine.

Four monkeys died 24 hours after receiving 1.7 mg/kg oxotremorine in the presence of sufficient atropine to completely prevent parasympathetic actions and effects on avoidance behavior. Atropine was also given after oxotremorine with the result that parasympathetic effects were not seen for 7 hrs after 1.7 mg/kg oxotremorine. Cause of death could not be determined by gross or microscopic pathological examination. In the absence of atropine, 0.3 mg/kg oxotremorine produced profuse parasympathetic symptoms and disrupted ongoing behavioral performances; however, recovery was complete within a few hours. Dose-effect curves for the other drugs used in this experiment were, therefore, not extended beyond doses which were safe when given alone.

Oxotremorine-M or neostigmine produced dose-related decreases in rates of responding and shock delivery with corresponding increases in response durations. In addition, after doses of 0.03 mg/kg (oxotremorine-M) or 0.1 mg/kg (neostigmine) and greater, the monkeys exhibited profuse urination, defecation, and salivation. All of the observed effects of oxotremorine-M or neostigmine were completely prevented by methylatropine (FIG. 2). Bethanechol only moderately decreased responding at 3 mg/kg, a dose which produced pronounced peripheral parasympathetic actions (including emesis), all effects of bethanechol were also prevented by methylatropine (data not shown).

Although methlylatropine completely prevented the peripheral manifestations, except emesis in one case, it did not influence the behavioral effects of oxotremorine or physostigmine (FIG. 3).

FIGURE 1. Effects of oxotremorine or physostigmine alone (○) and in combination with 1 mg/kg atropine (●). Although atropine produced pronounced effects on behavior when given alone (□), effects of oxotremorine and of physostigmine were generally prevented by atropine. However, response rates (top panels) were markedly increased above control values when atropine was given with either of these drugs. When atropine was given with physostigmine, rates of shock delivery were not completely returned to control levels. Points above S represent effects of saline given before the 10 min avoidance period following 0.3 mg/kg physostigmine. Escape failures are indicated by an *. Drug effects are means of at least one determination in each of 8 monkeys (5 for oxotremorine-atropine combinations).

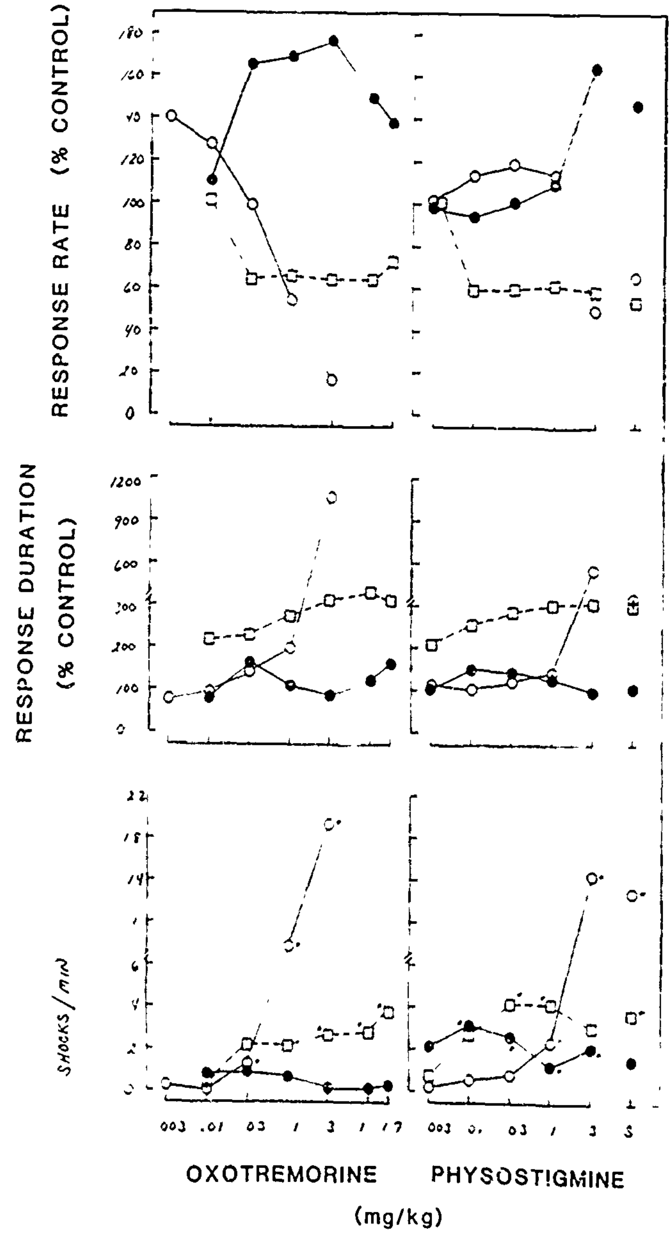


FIGURE 2. Effects of oxotremorine-M or neostigmine alone (○) and in combination with 1 mg/kg methylatropine (●). Methylatropine had no effect of its own (□) but completely prevented effects of oxotremorine-M or neostigmine. However, the combination of methylatropine with these compounds resulted in rates of responding that were greater than control values. Points above S represent effects of saline given before the 10 min avoidance period following either 0.1 mg/kg oxotremorine-M or 0.3 mg/kg neostigmine. Escape failures are indicated by an *. Drug effects are means of at least one determination in each of 4 monkeys.

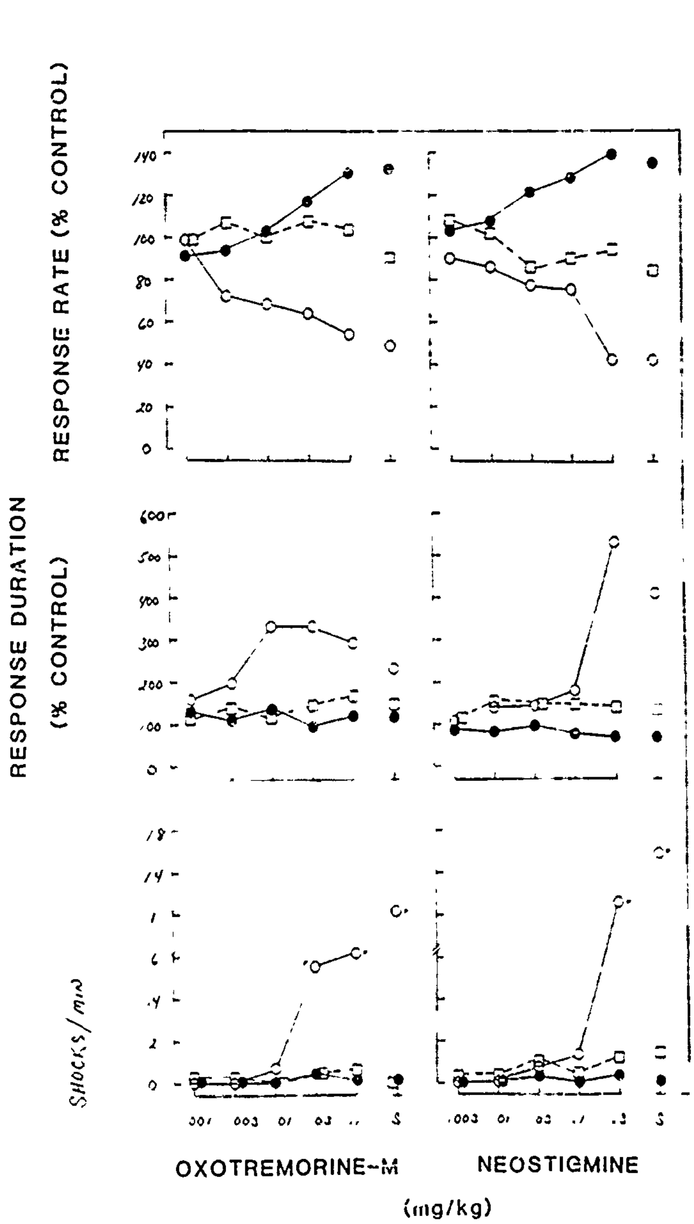
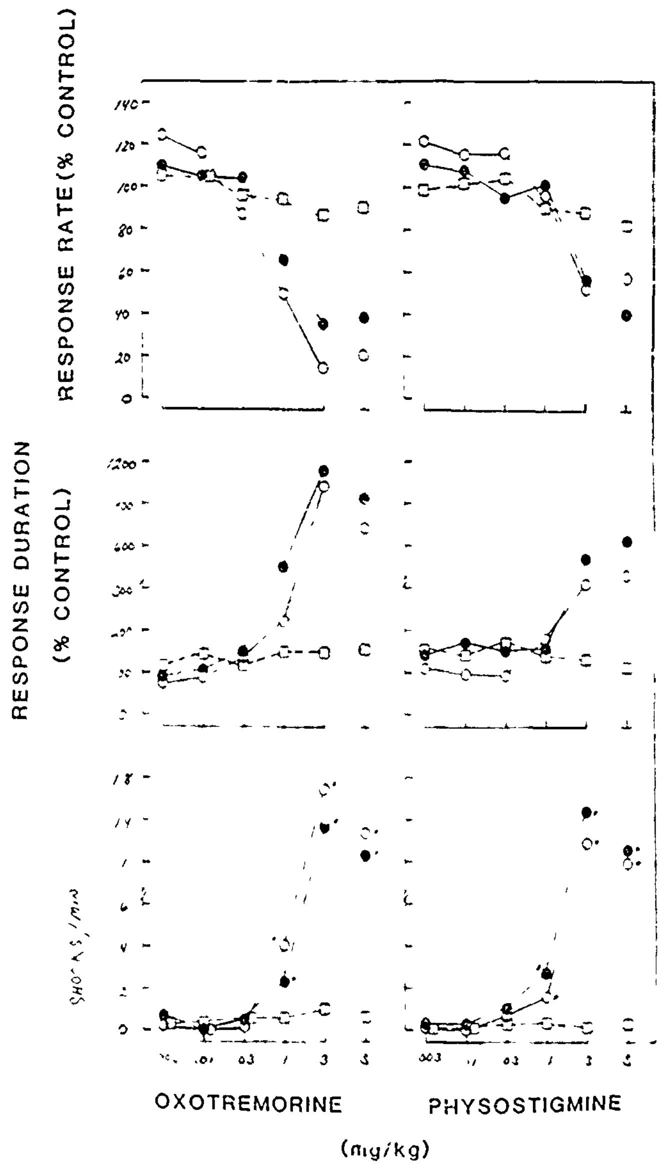


FIGURE 3. Effects of oxotremorine or physostigmine alone (○) and in combination with 1 mg/kg methylatropine (●). Methylatropine had little effect when given alone (□) and did not alter the dose-effect functions for either oxotremorine or physostigmine. Points above S represent effects of saline given before the 10 min avoidance period following 0.3 mg/kg oxotremorine or physostigmine. Escape failures are indicated by an *. Drug effects are means of at least one determination in each of 8 monkeys.



DISCUSSION

The similarity in the behavioral effects of physostigmine and neostigmine and between oxotremorine and oxotremorine-M suggest that peripheral parasympathetic actions of these compounds are sufficient to alter avoidance behavior. Methylatopine (1 mg/kg) demonstrated marked peripheral muscarinic receptor blockade in its actions against the quaternary compounds oxotremorine-M, neostigmine, and bethanechol. However, when methylatropine was given in conjunction with oxotrmorine or physostigmine, behavioral effects of these compounds were not reversed. The central actions of oxotremorine and physostigmine suggested by this observation were further documented by the reversal of the behavioral effects of both oxotremorine and physostigmine by atropine. Moreover, the methylatropine drug-interaction experiments revealed that this central component of action may be of overriding significance in the behavioral effects of oxotremorine or physostigmine since blockade of the peripheral muscarinic actions of these compounds was not sufficient to alter their behavioral effects. Taken as a whole, the data indicate that physostigmine produces behavioral effects primarily through actions at central muscarinic receptors.

Response rate-increases found after combinations of atropine with rate-decreasing doses of oxotremorine or physostigmine and after combinations of methylatropine with rate-decreasing doses of oxotremorine-M or neostigmine were surprising. Although small increases in response rate were seen with either oxotremorine or physostigmine alone, increases were not seen with the quaternary analogs. Thus, unmasking of agonist actions of the cholinomimetics by atropine seems unlikely.

We have observered possible non-muscarinic stimulant actions of atropine on behavior of squirrel monkeys studied under different experimental conditions (FED PROCEEDINGS 44 877, 1985). If such non-muscarinic actions of atropine also account for the rate increases reported here, then the interaction of the quaternary compounds with methylatropine suggest that the rate increases may be of peripheral origin.

In contrast, lethal effects of high doses of oxotremorine do not appear to be due to atropine. Doses of atropine 10 times higher have been given without any health effects. Moreover, results of experiments with oxotremorine in rats using several antimuscarinics, over a wide range of doses, implicate oxotremorine in lethality (see POSTER # 87).

Study of drug effects at the integrated level of behavior can often reveal drug actions which are not apparent under other systems. The absence of marked behavioral activity of bethanechol, for example, was unexpected based upon the peripheral muscarinic activity of this compound. Likewise, atropine did not completely prevent all behavioral effects of physostigmine (eg. effects on shock rates) in contrast to the physiological antagonism found in many tissue preparations.

MICROCLIMATE COOLING AND THE AIRCREW CHEMICAL DEFENSE ENSEMBLE

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US Army Aeromedical Research Laboratory, Fort Rucker, AL 36362-5000

QUESTIONS

1. Can Aviators maintain present duty schedules in MOPP IV Gear?

2. Does cooling make a difference to aviators in MOPP Gear?

HEAT STRESS STUDY

* Field Conditions
* 6 Day Duration
* Cooling/No Cooling

HEAT STRESS STUDY

* 6 Hours of Flight Daily
* Preflight thru Flight
* 12 Hour MOPP IV, 12 Hour MOPP I

SUBJECTS

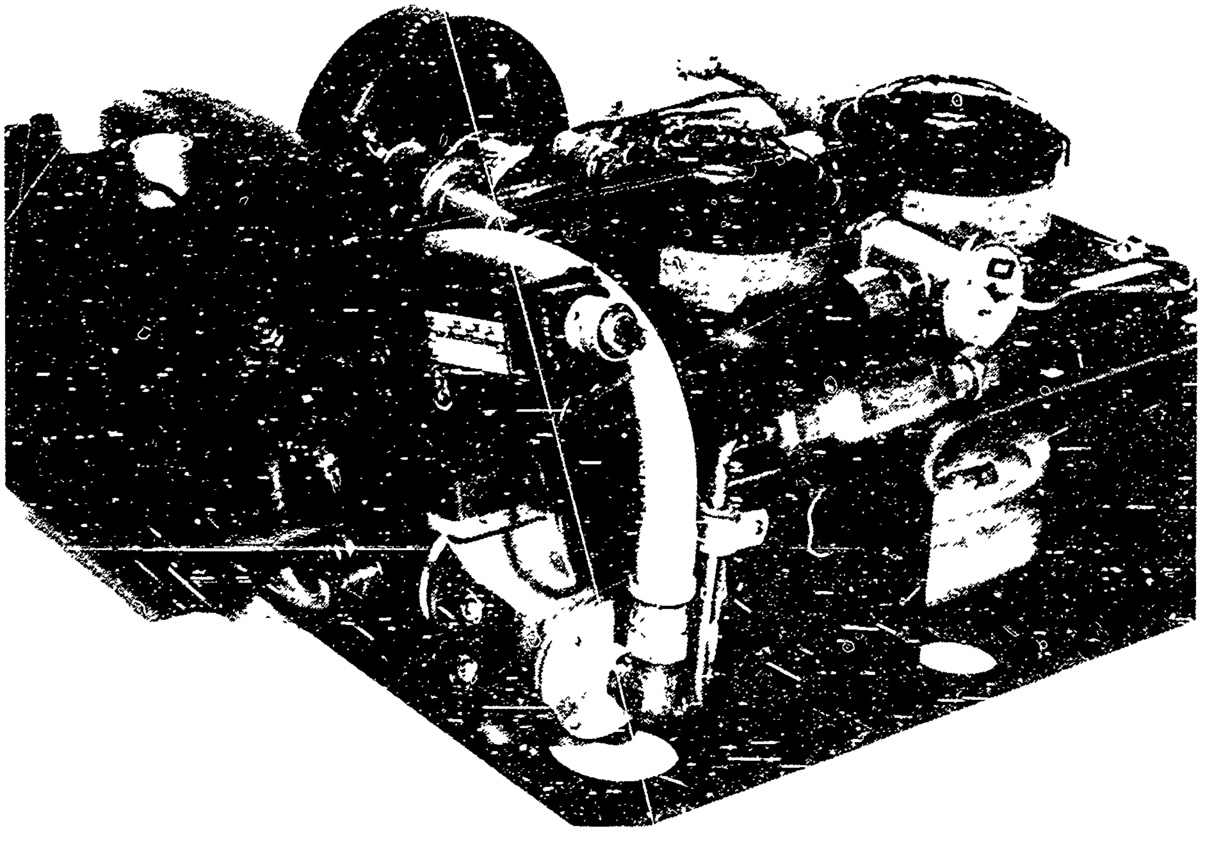
* Volunteers
* Operational
* 350 - 1800 Hours

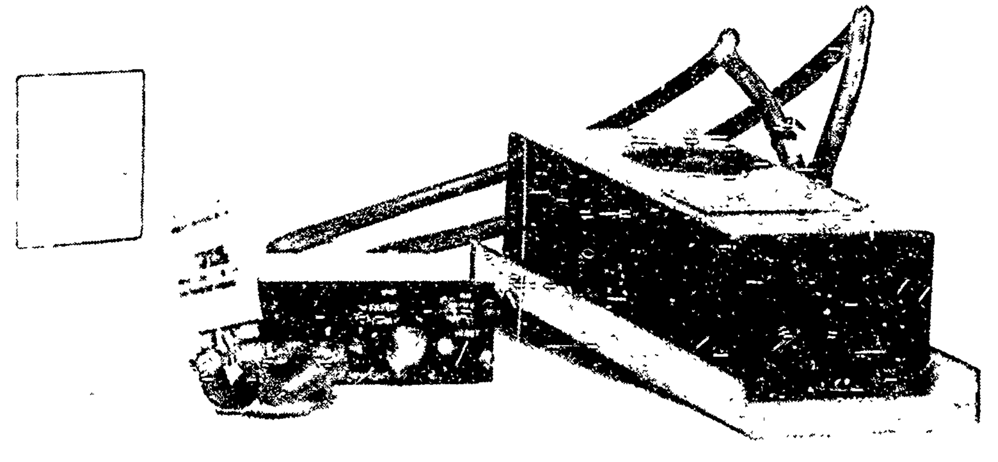
FLIGHT PROFILES

* HAATS
* Contour
* NOE
* GCA/ILS
* Recon
* Precision

Medical Test Summary

Subject No.	Age (yrs)	Vo2(max) mL/Kg/min	Oxygen pulse Vo2(max)/FC	Body fat (%)
1	31	40.33	19.68	19
2	33	38.02	16.09	27
3	32	37.47	18.31	22
5	25	42.65	14.02	24
6	29	45.72	19.07	22
7	32	43.55	20.43	20





A-1313

Actual Missions Flown

Subject	Day 1		Day 2		Day 3		Day 4		Day 5		Day 6	
1	NC	3*	NC	4	A1	2#	A1	4	A1	2#	NC	4
2	NC	3#	NC	4	A1	4	A1	@	A1	4	NC	3*
3	A1	4	A1	4	NC	4	NC	2#	A1	3#	A1	3#
5	NC	3*	NC	4	LQ	4	LQ	4	NC	2*	NC	2*
6	NC	3*$	NC	2*$	LQ	4$	LQ	4$	NC	4	NC	2*$
7	NC	2*$	NC	3*$	A2	3!$	A2	3@$	NC	4	NC	2*$

* Terminated for medical condition
\# Terminated for injury by equipment
@ Terminated for weather conditions
! Terminated for equipment problems
$ Doors Closed During Flight

NC = NO VEST
A1 = AIR VEST 1
A2 = AIR VEST 2
LQ = LIQUID VEST

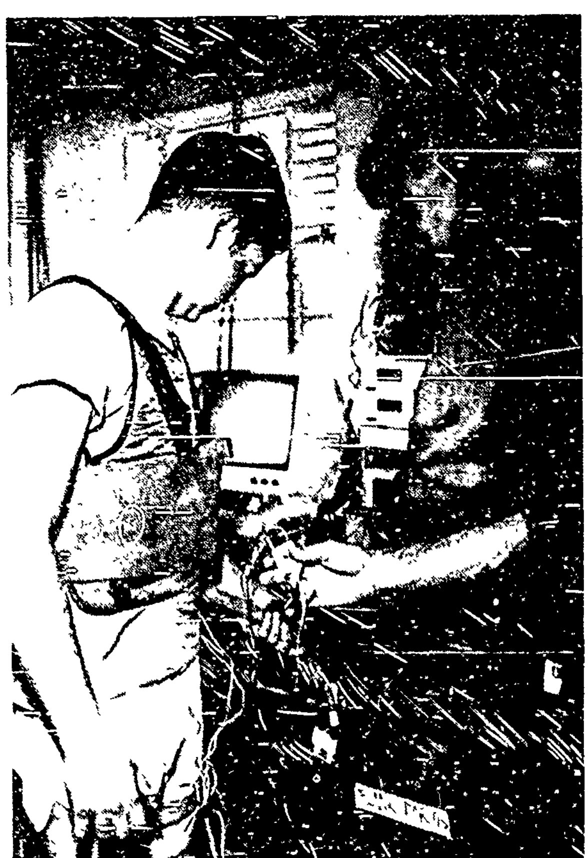
MOPP GEAR PROBLEMS

* Mask - "Hot Spots"
* Mask - Vision
* Mask - Drinking
* Mask - Breathing Resistance
* Mask - Cool Air
* Gloves
* Boots

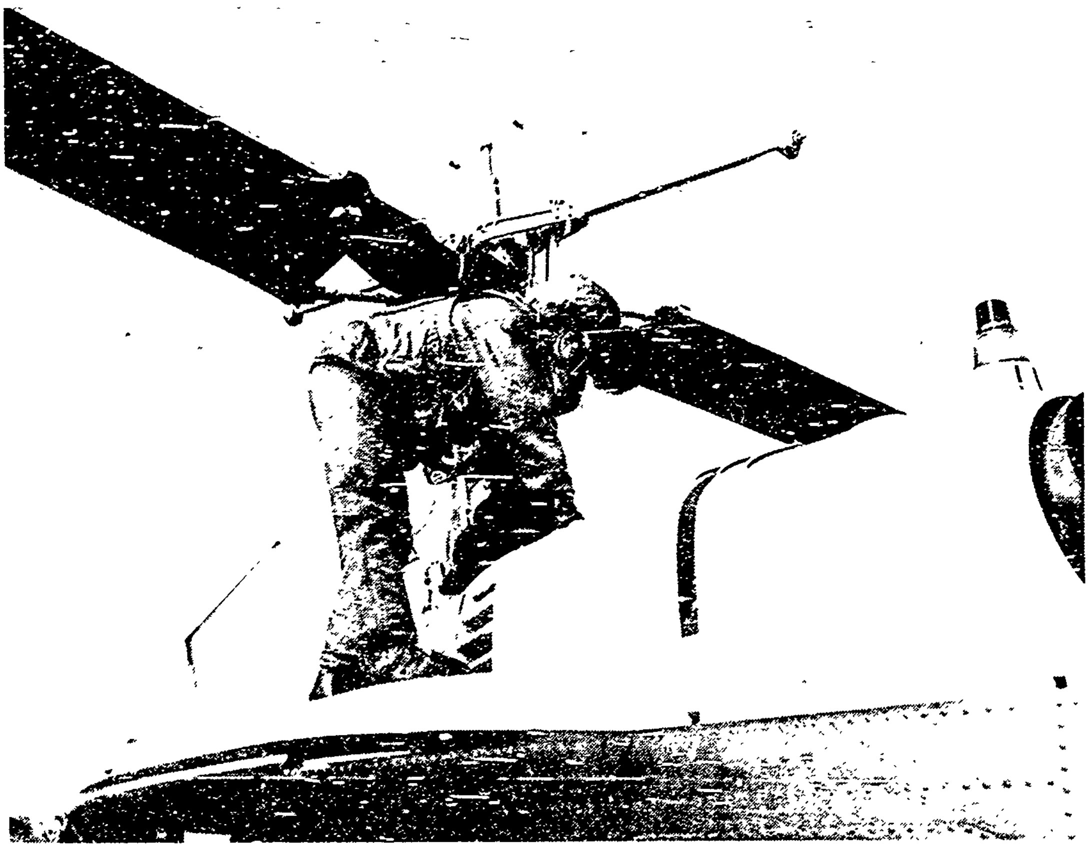
A-1315

COOLING SYSTEM PROBLEMS

* Leaks
* Bubbles
* Purging
* Ensemble Interface
* Flow

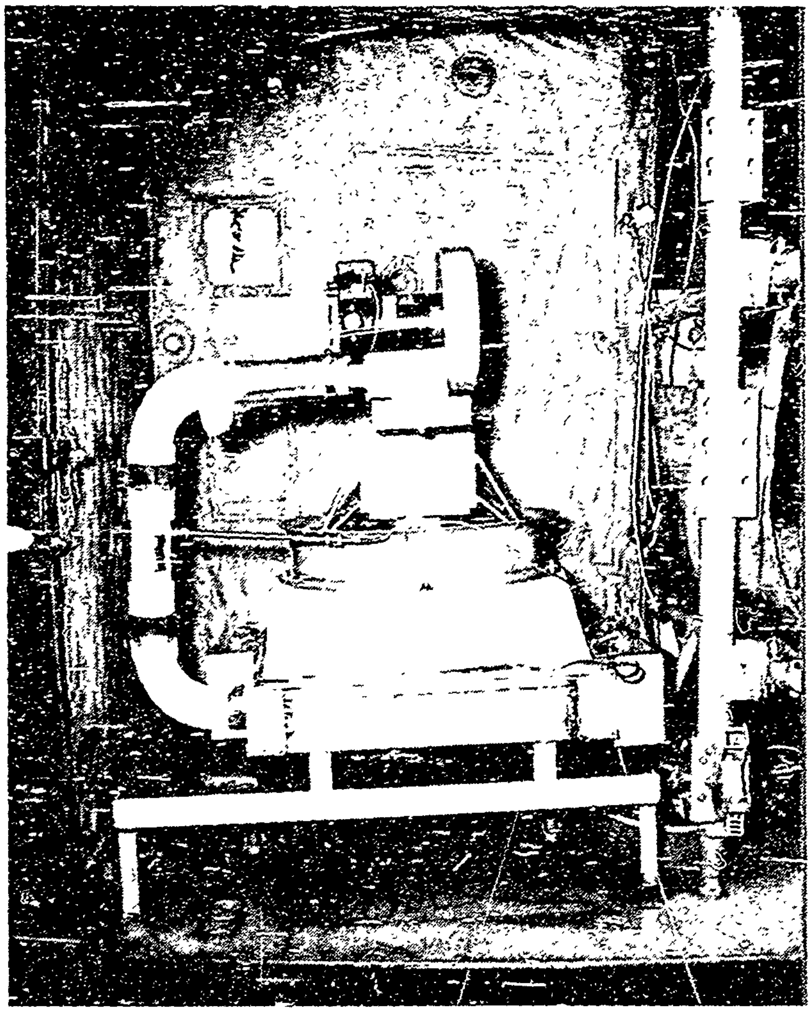


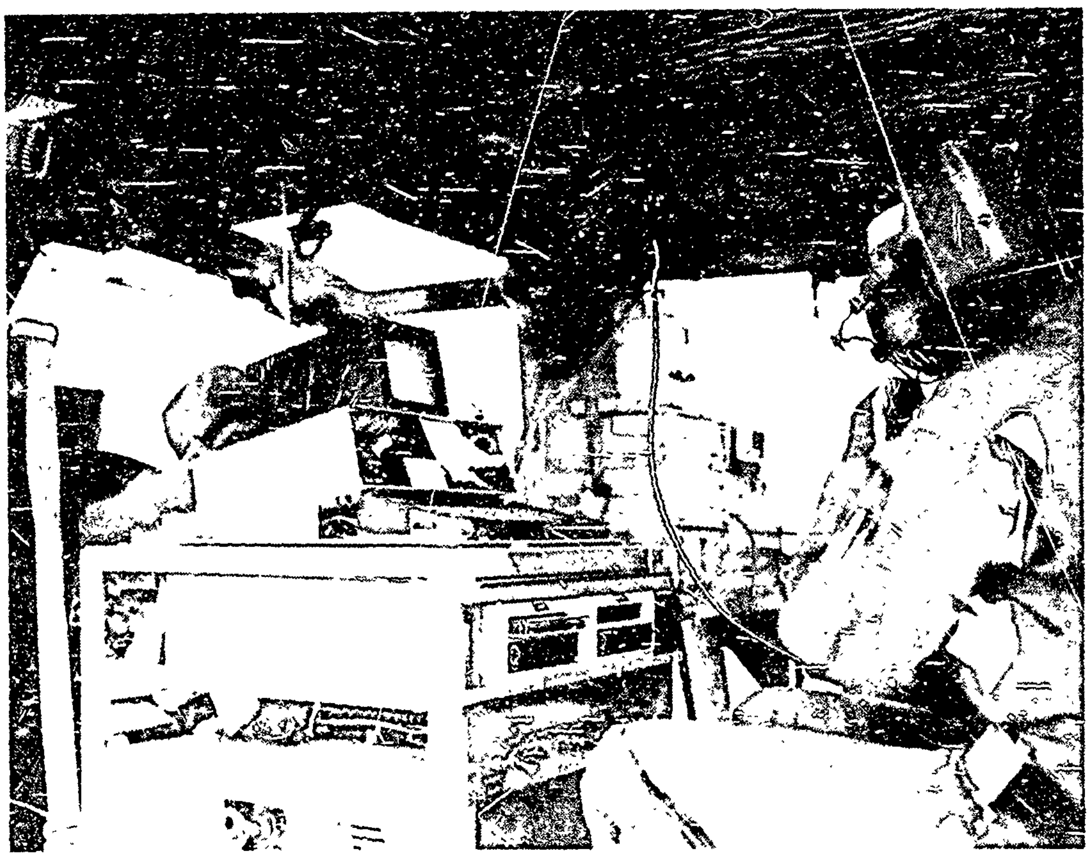
A-1318



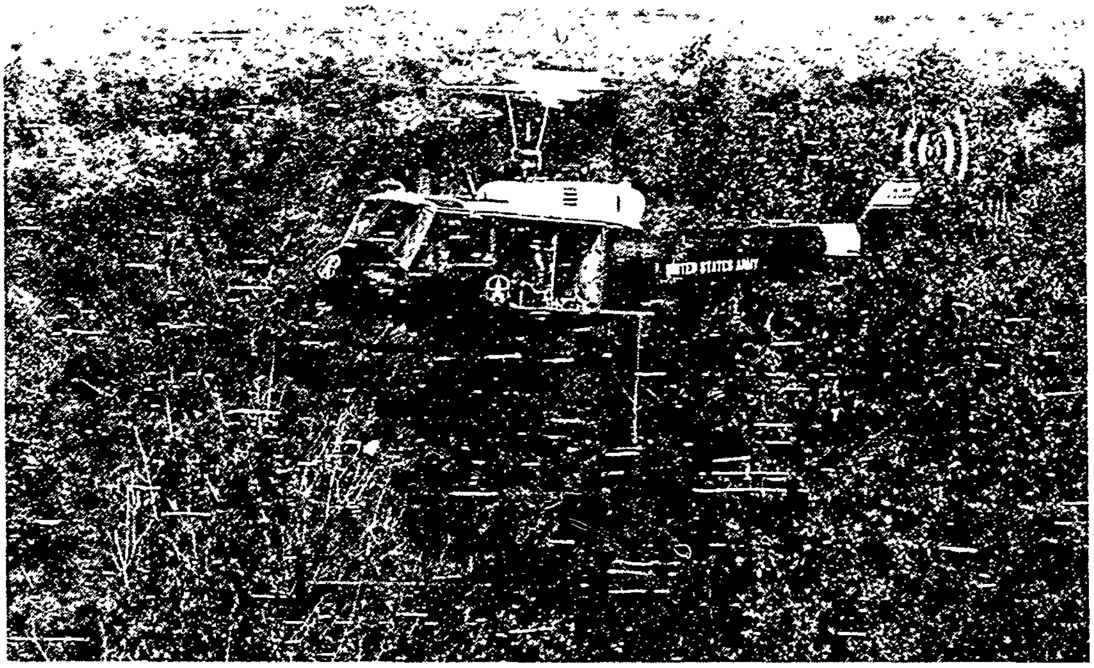
Typical Daily Schedule

0600	
0630	Wake up; clean up; breakfast
0700	Monitoring equipment hook up; MOPP IV
0730	
0800	PAB/ZITA testing
0830	
0900	Preflight check; plan Mission I
0930	
1000	Mission I
1030	
1100	Refuel; plan Mission II
1130	
1200	Mission II
1230	
1300	Refuel; lunch; plan Mission III
1330	
1400	Mission III
1430	
1500	Refuel; plan Mission IV
1530	
1600	Mission IV
1630	
1700	PAB/ZITA testing
1730	
1800	Daily debriefing; shower
1830	
1900	MOPP I; dinner
1930	
2000	Free time
2030	
2100	Sleep





A-1320



ALL COOL VESTS

PREVENTED OVERHEATING

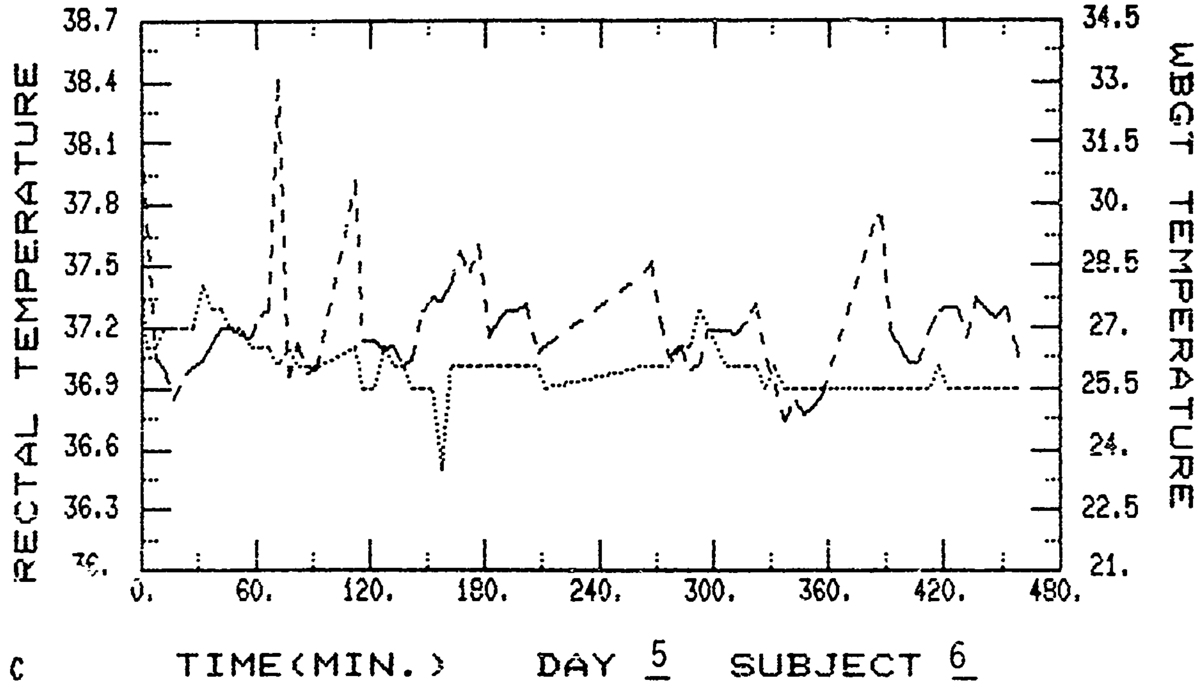
COCKPIT TEMPERATURES
BELOW 29°C WBGT

* Not Overheated with Cooling

* Not Overheated Without Cooling

A-1321

Doors Open
Pilot Not Wearing Cool Vest

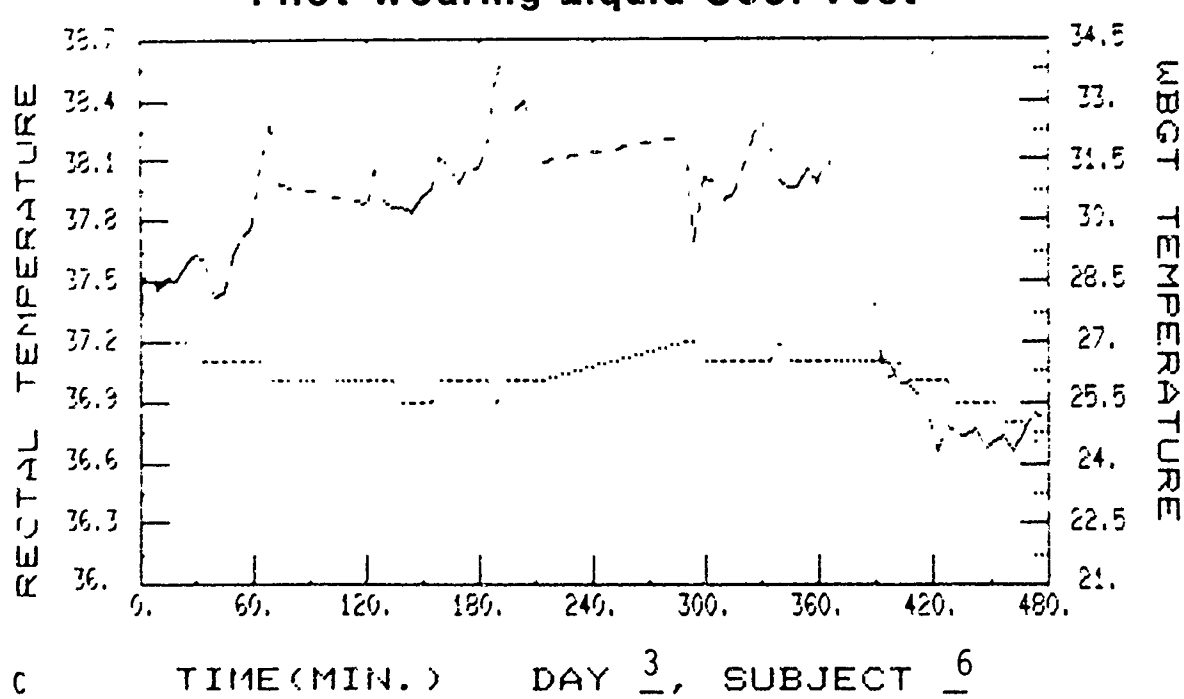


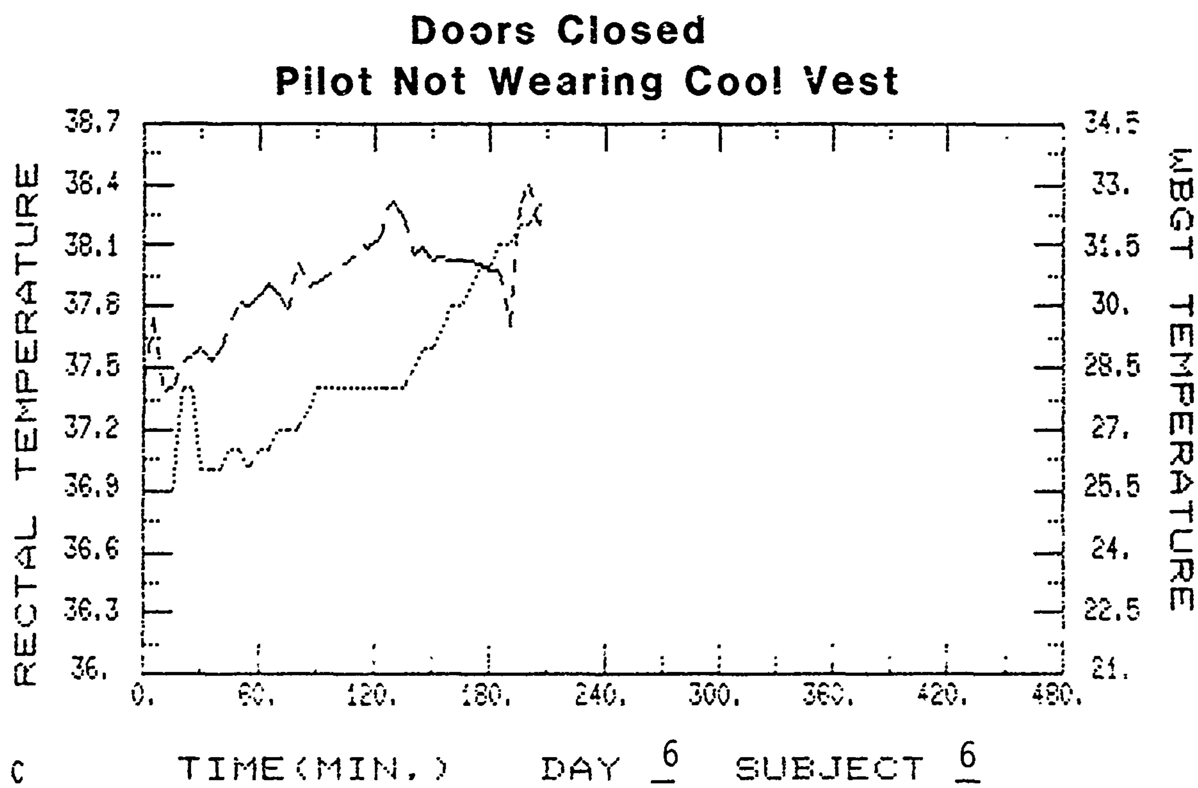
COCKPIT TEMPERATURES
ABOVE 29°C WBGT

* Not Overheated With Cooling

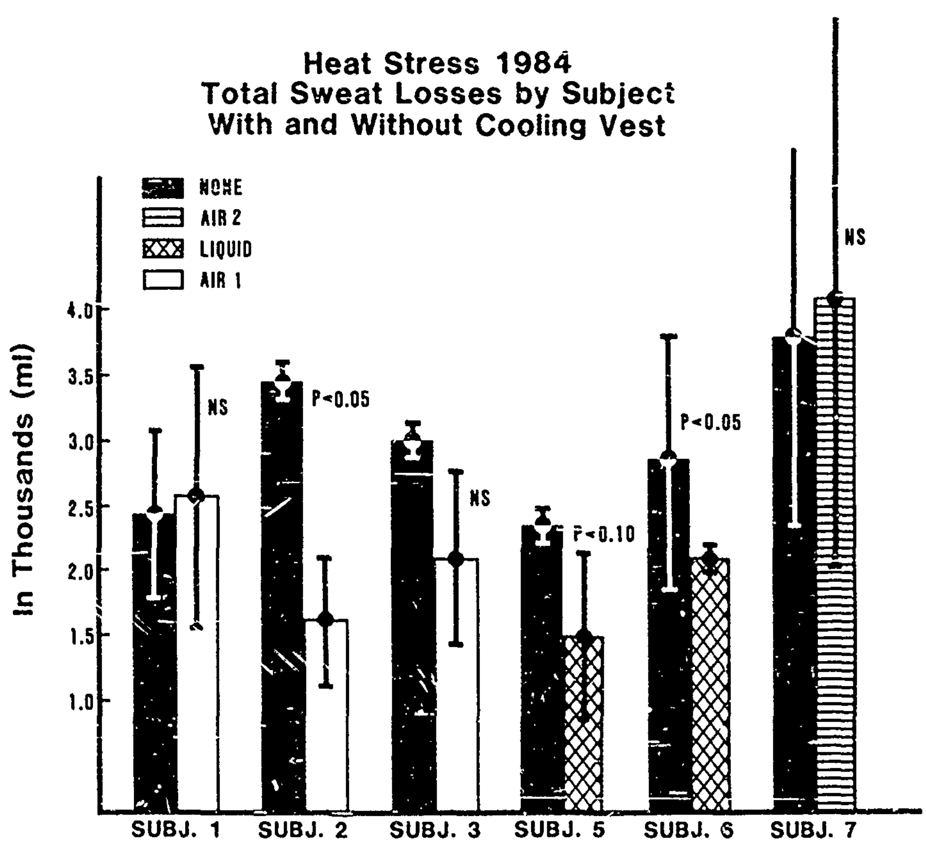
* Overheats Without Cooling

Doors Closed
Pilot Wearing Liquid Cool Vest




Doors Closed
Pilot Not Wearing Cool Vest
RECTAL TEMPERATURE
WBGT TEMPERATURE
38.7
38.4
38.1
37.8
37.5
37.2
36.9
36.6
36.3
36.
33.
31.5
30.
28.5
27.
25.5
24.
22.5
21.
0.
60.
120.
180.
240.
300.
360.
420.
480.
TIME(MIN.) DAY 6 SUBJECT 6

C


Heat Stress 1984
Total Sweat Losses by Subject
With and Without Cooling Vest
NONE
AIR 2
LIQUID
AIR 1
In Thousands (ml)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
NS
P<0.05
NS
P<0.10
P<0.05
NS
SUBJ. 1
SUBJ. 2
SUBJ. 3
SUBJ. 5
SUBJ. 6
SUBJ. 7

CARDIOVASCULAR PHYSIOLOGY

* Heart Rate Correlated With Rectal Temperature
* Serum Electrolytes Normal Throughout
* Urine Specific Gravity Variable Daily
* 1 to 2% Overall Weight Loss for Week

ZITA

* Multi Task Psychomotor Task
* Learning Effects Present
* AM/PM Decrease in Performance When Hot

PAB

* Computerized Psychological Assessment Battery
* Depression Score Increased With Core Temperature
* Cognitive Score Not Correlated With Temperature
* Wilkinson 4-Choice Task Correlated With Temperature

HIMS II

* Records Aircraft Flight Parameters
* Records Subject Control Inputs
* No Correlation With Temperature Found

SUBJECTIVE RATINGS

* Evaluation By Safety Pilots
* Scoring Based on ATM Standards
* No Difference Found With Temperature

COMMENTS

* Outbrief Questionnaire
* Increased Training Wanted
* Improved MOPP Gear Wanted
* All Subjects Wanted Cooling Vests

MICROCOMPUTER PROGRAM FOR SIMULATION OF HEAT STRESS

C.W. Mitchell, J. Rosario and F.S. Knox III
US Army Aeromedical Research Laboratory, Fort Rucker, AL 36362

```
*** U S A A R L ***
***THERMAL STRESS MODEL***
*** PHYSIOLOGICAL DATA***

PHYSICAL CHARACTERISTICS

SUBJECT'S HEIGHT IS 180 CM
SUBJECT'S WEIGHT IS 70 KG

FLIGHT VARIABLES

SUBJECT IS WEARING MOPP IV GEAR
AIRCRAFT IS A UH-1 HUEY HELICOPTER

SUBJECT HAS ACCLIMATIZED FOR 10 DAYS

----------------------------------------------------------------

THESE ARE THE BASELINE VALUES FOR PULSE AND RECTAL TEMPERATURE

TIME=  0              PULSE=  70             TEMP= 37

----------------------------------------------------------------------
----------------------------------------------------------------------

BELOW IS PHASE NUMBER 1 WHICH IS THE PREFLIGHT CHECK PORTION

THIS IS A CUSTOM ENVIRONMENT

AMBIENT AIR TEMP = 25 C
RELATIVE HUMIDITY = 50 %

AIR VELOCITY = 1 M/SEC
CLOUD DENSITY = 0 %

DURATION OF THIS PHASE = 45 MINUTES
METABOLIC RATE = 300 WATTS

-------------------------------------------------------------------

TIME=  5              PULSE= 102             TEMP= 37
TIME= 10              PULSE= 113             TEMP= 37.1
TIME= 15              PULSE= 121             TEMP= 37.1
TIME= 20              PULSE= 127             TEMP= 37.2
TIME= 25              PULSE= 131             TEMP= 37.3
TIME= 30              PULSE= 133             TEMP= 37.3
TIME= 35              PULSE= 134             TEMP= 37.3
TIME= 40              PULSE= 135             TEMP= 37.4
TIME= 45              PULSE= 136             TEMP= 37.4

----------------------------------------------------------------------
```

```
                      RECTAL TEMP (C)

    36                            39                            42
    !         !         !         !         !         !         !
  -----------------------------------------------------------------
 0 -I         *
-----------------------------------PREFLIGHT CHECK
 5 -I           *
10 -I           *
15 -I           *
20 -I           *
25 -I             *
30 -I             *
35 -I             *
40 -I             *
45 -I              *
-----------------------------------RECON
 5 -I             *
10 -I           *
15 -I           *
20 -I         *
25 -I         *
30 -I         *
35 -I         *
40 -I         *
45 -I         *
50 -I        *
55 -I        *
60 -I        *
-----------------------------------FARP ACTIVITY
 5 -I        *
10 -I         *
15 -I         *
20 -I         *
25 -I           *
30 -I           *
-----------------------------------MISSION COMPLETE
```

```
                           HEART RATE (BPM)

     0                           90                            180
     !              !              !              !              !
  -----------------------------------------------------------------
 0 -I                      *
-----------------------------------PREFLIGHT CHECK
 5 -I                                *
10 -I                                    *
15 -I                                       *
20 -I                                         *
25 -I                                          *
30 -I                                           *
35 -I                                           *
40 -I                                            *
45 -I                                            *
-----------------------------------RECON
 5 -I                                  *
10 -I                               *
15 -I                             *
20 -I                           *
25 -I                          *
30 -I                          *
35 -I                         *
40 -I                         *
45 -I                         *
50 -I                         *
55 -I                         *
60 -I                         *
-----------------------------------FARP ACTIVITY
 5 -I                          *
10 -I                          *
15 -I                          *
20 -I                          *
25 -I                          *
30 -I                          *
-----------------------------------MISSION COMPLETE
```

*** U S A A R L ***
THERMAL STRESS MODEL
*** PHYSIOLOGICAL DATA***

PHYSICAL CHARACTERISTICS

SUBJECT'S HEIGHT IS 180 CM
SUBJECT'S WEIGHT IS 70 KG

FLIGHT VARIABLES

SUBJECT IS WEARING A STD FLIGHT SUIT
AIRCRAFT IS A UH-1 HUEY HELICOPTER

SUBJECT HAS ACCLIMATIZED FOR 10 DAYS

THESE ARE THE BASELINE VALUES FOR PULSE AND RECTAL TEMPERATURE

TIME= 0	PULSE= 70	TEMP= 37

BELOW IS PHASE NUMBER 1 WHICH IS THE PREFLIGHT CHECK PORTION

THIS IS A SUMMER ENVIRONMENT

AMBIENT AIR TEMP = 35 C
RELATIVE HUMIDITY = 45 %

AIR VELOCITY = 1 M/SEC
CLOUD DENSITY = 0 %

DURATION OF THIS PHASE = 45 MINUTES
METABOLIC RATE = 300 WATTS

TIME= 5	PULSE= 103	TEMP= 37
TIME= 10	PULSE= 115	TEMP= 37.1
TIME= 15	PULSE= 124	TEMP= 37.2
TIME= 20	PULSE= 130	TEMP= 37.2
TIME= 25	PULSE= 134	TEMP= 37.3
TIME= 30	PULSE= 136	TEMP= 37.4
TIME= 35	PULSE= 138	TEMP= 37.4
TIME= 40	PULSE= 139	TEMP= 37.4
TIME= 45	PULSE= 139	TEMP= 37.5

BELOW IS PHASE NUMBER 2 WHICH IS THE RECON PORTION

THIS IS A CUSTOM ENVIRONMENT

AMBIENT AIR TEMP = 20 C
RELATIVE HUMIDITY = 45 %

AIR VELOCITY = 1 M/SEC
CLOUD DENSITY = 0 %

DURATION OF THIS PHASE = 60 MINUTES
METABOLIC RATE = 120 WATTS

TIME= 5	PULSE= 107	TEMP= 37.4
TIME= 10	PULSE= 96	TEMP= 37.3
TIME= 15	PULSE= 90	TEMP= 37.2
TIME= 20	PULSE= 86	TEMP= 37.1
TIME= 25	PULSE= 83	TEMP= 37.1
TIME= 30	PULSE= 81	TEMP= 37
TIME= 35	PULSE= 80	TEMP= 37
TIME= 40	PULSE= 79	TEMP= 37
TIME= 45	PULSE= 79	TEMP= 37
TIME= 50	PULSE= 78	TEMP= 36.9
TIME= 55	PULSE= 78	TEMP= 36.9
TIME= 60	PULSE= 78	TEMP= 36.9

BELOW IS PHASE NUMBER 3 WHICH IS THE FARP ACTIVITY PORTION

THIS IS A CUSTOM ENVIRONMENT

AMBIENT AIR TEMP = 28 C
RELATIVE HUMIDITY = 50 %

AIR VELOCITY = 1 M/SEC
CLOUD DENSITY = 75 %

DURATION OF THIS PHASE = 30 MINUTES
METABOLIC RATE = 105 WATTS

TIME= 5	PULSE= 81	TEMP= 36.9
TIME= 10	PULSE= 82	TEMP= 37
TIME= 15	PULSE= 83	TEMP= 37
TIME= 20	PULSE= 83	TEMP= 37.1
TIME= 25	PULSE= 83	TEMP= 37.2
TIME= 30	PULSE= 84	TEMP= 37.2

MISSION COMPLETE

BELOW IS PHASE NUMBER 2 WHICH IS THE RECON PORTION

THIS IS A SUMMER ENVIRONMENT

AMBIENT AIR TEMP = 35 C
RELATIVE HUMIDITY = 45 %

AIR VELOCITY = 1 M/SEC
CLOUD DENSITY = 0 %

DURATION OF THIS PHASE = 60 MINUTES
METABOLIC RATE = 125 WATTS

TIME= 5	PULSE= 117	TEMP= 37.5
TIME= 10	PULSE= 108	TEMP= 37.5
TIME= 15	PULSE= 102	TEMP= 37.4
TIME= 20	PULSE= 98	TEMP= 37.4
TIME= 25	PULSE= 95	TEMP= 37.4
TIME= 30	PULSE= 93	TEMP= 37.3
TIME= 35	PULSE= 92	TEMP= 37.3
TIME= 40	PULSE= 91	TEMP= 37.3
TIME= 45	PULSE= 90	TEMP= 37.2
TIME= 50	PULSE= 90	TEMP= 37.2
TIME= 55	PULSE= 89	TEMP= 37.2
TIME= 60	PULSE= 89	TEMP= 37.1

BELOW IS PHASE NUMBER 3 WHICH IS THE FARP ACTIVITY PORTION

THIS IS A SUMMER ENVIRONMENT

AMBIENT AIR TEMP = 35 C
RELATIVE HUMIDITY = 45 %

AIR VELOCITY = 1 M/SEC
CLOUD DENSITY = 75 %

DURATION OF THIS PHASE = 30 MINUTES
METABOLIC RATE = 105 WATTS

TIME= 5	PULSE= 87	TEMP= 37.2
TIME= 10	PULSE= 86	TEMP= 37.2
TIME= 15	PULSE= 85	TEMP= 37.2
TIME= 20	PULSE= 84	TEMP= 37.2
TIME= 25	PULSE= 84	TEMP= 37.2
TIME= 30	PULSE= 83	TEMP= 37.2

MISSION COMPLETE

```
                      RECTAL TEMP (C)

    36                            39                            42
    !         !         !         !         !         !         !
  ------------------------------------------------------------------
 0 -I         *
-----------------------------------PREFLIGHT CHECK
 5 -I           *
10 -I           *
15 -I             *
20 -I              *
25 -I              *
30 -I                *
35 -I                *
40 -I                *
45 -I                  *
-----------------------------------RECON
 5 -I                *
10 -I                *
15 -I                *
20 -I              *
25 -I              *
30 -I              *
35 -I              *
40 -I             *
45 -I             *
50 -I             *
55 -I             *
60 -I           *
-----------------------------------FARP ACTIVITY
 5 -I           *
10 -I           *
15 -I           *
20 -I           *
25 -I           *
30 -I           *
-----------------------------------MISSION COMPLETE
```

```
                           HEART RATE (BPM)

     0                           90                           180
     !              !              !              !              !
  ------------------------------------------------------------------
 0 -I                      *
-----------------------------------PREFLIGHT CHECK
 5 -I                                   *
10 -I                                       *
15 -I                                          *
20 -I                                             *
25 -I                                               *
30 -I                                                *
35 -I                                                 *
40 -I                                                  *
45 -I                                                  *
-----------------------------------RECON
 5 -I                                          *
10 -I                                       *
15 -I                                     *
20 -I                                    *
25 -I                                   *
30 -I                                  *
35 -I                                 *
40 -I                                 *
45 -I                                 *
50 -I                                *
55 -I                                *
60 -I                                *
-----------------------------------FARP ACTIVITY
 5 -I                               *
10 -I                              *
15 -I                              *
20 -I                             *
25 -I                             *
30 -I                             *
-----------------------------------MISSION COMPLETE
```

ASSESSING RELATIVE ANTICHOLINERGIC AND ANTICHOLINESTERASE POTENCY OF DRUGS USING HEAT-STRESSED ATROPINIZED RATS

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ABSTRACT

HUMAN VOLUNTEERS ARE OF LIMITED USE IN TESTING THE EFFECTS OF ATROPINE IN HOT ENVIRONMENTS BECAUSE ATROPINE, BY INHIBITING SWEATING AND INCREASING HEART RATE, INCREASES THE RISK OF HEATSTROKE. HEAT-EXPOSED RATS SPREAD SALIVA OVER THEIR BODIES FOR EVAPORATIVE COOLING; THIS PROCESS IS CHOLINERGICALLY REGULATED JUST AS SWEAT SECRETION IS IN HUMANS. ATROPINE, IN DOSES ANALOGOUS TO THOSE GIVEN TO MAN AS NERVE AGENT ANTIDOTES, RESULTED IN A FOUR-FOLD INCREASE IN HEATING RATE IN OUR HEAT-STRESSED RAT MODEL. IT IS IMPORTANT TO KNOW THE EFFECTS OF ANTICHOLINERGIC DRUGS ON COOLING ABILITY IN ORDER TO DETERMINE WHICH DRUGS OR DOSES WOULD BE CONTRAINDICATED FOR USE IN HOT ENVIRONMENTS. ALSO, SINCE ATROPINE ADMINISTRATION RESULTS IN AN INCREASE IN THE RATE OF HEATING, A REVERSAL OF ITS EFFECT BY CARBAMATE ADMINISTRATION MAY BE INDICATIVE OF RELATIVE ANTICHOLINESTERASE ACTIVITY.

IN THESE EXPERIMENTS, ADULT MALE SPRAGUE-DAWLEY RATS (510-530g) WERE INJECTED (TAIL VEIN) WITH THE APPROPRIATE DRUG 15 MINUTES PRIOR TO THE START OF THE HEAT-STRESS. THE RATS WERE PLACED UNRESTRAINED IN A 41.5°C CHAMBER UNTIL A COLONIC TEMPERATURE OF 42.6°C WAS REACHED; THEN THEY WERE REMOVED TO A 26°C CHAMBER TO COOL. OF THE VARIABLES MEASURED HEATING RATE (°C/MIN) WAS THE MOST SENSITIVE INDEX OF DRUG ACTIVITY.

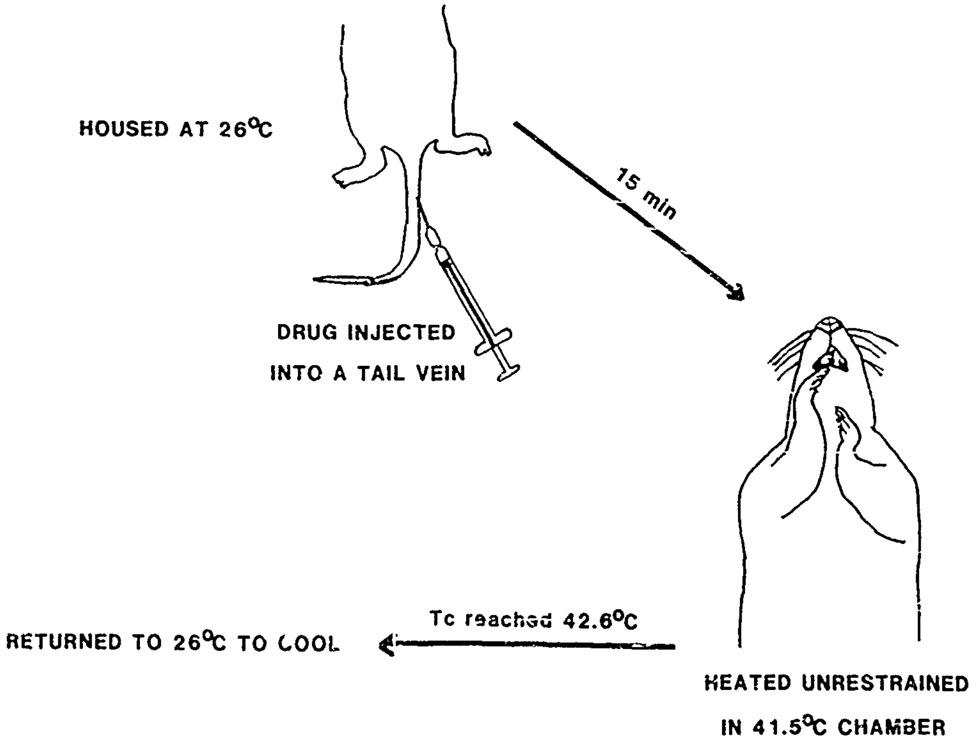
BECAUSE OF THE SENSITIVITY OF EFFECTS ON THERMOREGULATION, WE PLOTTED THE NATURAL LOG OF THE DOSE OF ATROPINE VERSUS THE HEATING RATES OF 7 GROUPS OF 12 RATS (10-1000 ug/kg OF ATROPINE). THE HEATING RATE WAS LINEARLY RELATED TO THE DOSE OVER THIS RANGE. USING THE HEATING RATES ELICITED BY THE ANTICHOLINERGIC DRUGS INDICATED BELOW AND THE REGRESSION EQUATION OF ATROPINE DOSE VS. HEATING RATE, WE DETERMINED AN EQUIVALENT ATROPINE DOSE FOR EACH DRUG DOSE. USING A RATIO OF THE EQUIVALENT DOSE TO THE ACTUAL DOSE, WE CALCULATED A POTENCY RATIO OF EACH DRUG RELATIVE TO ATROPINE AS FOLLOWS: IMIPRAMINE (0.004), AMITRIPTYLINE (0.02), CHLORPROMAZINE (0.1), ATROPINE (1), L-HYOSCYAMINE (2), ATROPINE METHYL NITRATE (4), AND SCOPOLAMINE (16).

TO ASSESS THE ANTICHOLINESTERASE ACTIVITY OF 3 CARBAMATES, 200 ug/kg OF ATROPINE WAS GIVEN 30 MINUTES PRIOR TO THE START OF HEAT-STRESS, FOLLOWED IN 15 MINUTES BY PHYSOSTIGMINE, PYRIDOSTIGMINE OR NEOSTIGMINE. IF QUANTITIES OF THE CARBAMATES ARE CONVERTED TO UMOLES OF FREE BASE PRODUCING EQUIVALENT CHANGES IN THE ATROPINE-INDUCED HEATING RATE, THEN A RELATIVE POTENCY AMONG THE 3 DRUGS CAN BE DETERMINED. WE DEMONSTRATED THE FOLLOWING RELATIVE POTENCY VALUES: PYRIDOSTIGMINE (1). PHYSOSTIGMINE (4), AND NEOSTIGMINE (8). THEREFORE, THIS MODEL IS ALSO A PROMISING TOOL WITH WHICH TO EVALUATE THE INTENSITY OF THE ANTICHOLINESTERASE EFFECTS OF OTHER COMPOUNDS (NERVE AGENTS, INSECTICIDES.

INTRODUCTION

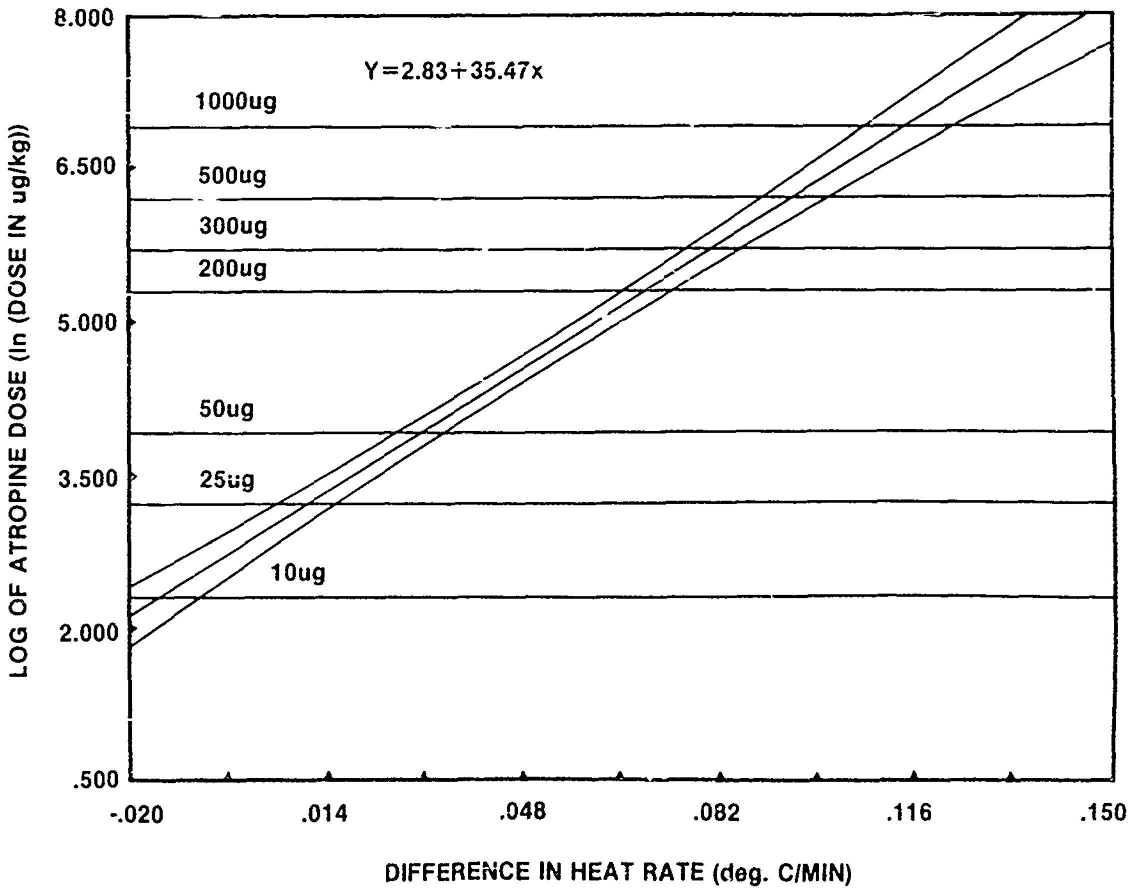
ANTICHOLINERGICS AND ANTICHOLINESTERASES HAVE EFFECTS THAT ARE NOT LIMITED TO A SINGLE SITE OF ACTION; THEREFORE, A WHOLE ORGANISM BIOASSAY HAS AN ADVANTAGE OVER ANY *IN VITRO* SYSTEM. WE HAVE PREVIOUSLY CONFIRMED THE SUITABILITY OF A HEAT-STRESSED RAT MODEL TO SIMULATE HUMAN RESPONSES IN HOT ENVIRONMENTS (J. APPL. PHYSIOL. 42, 809, 1977). HEAT EXPOSED RATS SPREAD SALIVA OVER THEIR BODIES FOR EVAPORATIVE COOLING; THIS PROCESS IS CHOLINERGICALLY REGULATED JUST AS SWEAT SECRETION IS IN HUMANS. THE EFFECTS OF DOSES OF ATROPINE FROM 10-4000 ug/kg WERE EXAMINED. (J. APPL. PHYSIOL. 53, 1171, 1982). HEATING RATE (°C/MIN) WAS THE MOST SENSITIVE INDEX OF DRUG ACTIVITY MEASURED. SIGNIFICANT ELEVATIONS IN HEATING RATE OVER SALINE CONTROLS WERE OBSERVED OVER A RANGE OF DOSES FROM 25-1000 ug/kg. AT A DOSE OF 200 ug/kg (EQUIVALENT TO 2 mg/70 kg HUMAN) SIGNIFICANT EFFECTS ON THERMOREGULATION PERSISTED FOR UP TO 3 HOURS AFTER ATROPINE ADMINISTRATION (IN PRESS). OTHER DRUGS, SUCH AS SCOPOLAMINE, ANTIHISTAMINES, TRANQUILIZERS, COLD MEDICATIONS AND ANTIDIARRHEAL MEDICATIONS, ARE SIMILAR TO ATROPINE IN THEIR ANTICHOLINERGIC PROPERTIES. IT IS IMPORTANT, THEREFORE, TO DETERMINE THE EFFECTS OF THESE MEDICATIONS ON COOLING ABILITY IN ORDER TO DETERMINE WHICH DRUGS OR DOSES WOULD BE CONTRAINDICATED FOR USE IN HOT ENVIRONMENTS. SINCE ATROPINE ADMINISTRATION RESULTS IN AN INCREASE IN THE RATE OF HEATING, A REVERSAL OF ITS EFFECT BY CARBAMATE ADMINISTRATION IS INDICATIVE OF ANTICHOLINESTERASE ACTIVITY.

FIG. 1
EXPERIMENTAL STRESS



ADULT, MALE SPRAGUE-DAWLEY RATS (510-530g) WERE USED. PRIOR TO HEAT STRESS, STERILE SALINE (0.2 ml) OR THE EXPERIMENTAL DRUG DISSOLVED IN AN EQUAL VOLUME OF SALINE WAS INJECTED INTO A LATERAL TAIL VEIN. THE RATS WERE HEAT-STRESSED UNRESTRAINED IN A CHAMBER MAINTAINED AT 41.5°C AND 30% RH UNTIL A CORE TEMPERATURE (Tc) OF 42.6°C WAS ATTAINED. THEN, THE ANIMALS WERE QUICKLY REMOVED FROM THE HEAT, WEIGHED AND ALLOWED TO PASSIVELY COOL AT 26°C. TO DETERMINE THE ANTICHOLINERGIC POTENCY OF OTHER DRUGS RELATIVE TO ATROPINE, THE DRUGS WERE GIVEN 15 MINUTES PRIOR TO THE START OF HEAT STRESS. THESE DRUG DOSES WERE CHOSEN TO APPROXIMATE THE EFFECTS OF 200 ug/kg OF ATROPINE. TO DETERMINE THE DEGREE OF REVERSAL OF ANTICHOLINERGIC INHIBITION OF SALIVATION BY THE SUBSEQUENT ADMINISTRATION OF ANTICHOLINESTERASES (CARBAMATES), ATROPINE (200 ug/kg) WAS GIVEN 30 MINUTES PRIOR TO THE HEAT STRESS, FOLLOWED 15 MINUTES LATER BY PHYSOSTIGMINE, PYRIDOSTIGMINE, OR NEOSTIGMINE PRIOR TO THE HEAT STRESS.

FIG. 2



SINCE THE HEATING RATE OF THE HEAT-STRESSED RAT WAS THE MOST SENSITIVE INDEX OF DRUG ACTIVITY, WE PLOTTED (FIG. 2) THE NATURAL LOG OF THE ATROPINE DOSE VERSUS THE GROUP HEATING RATES, CORRECTED FOR THE AVERAGE HEATING RATE OF SALINE CONTROLS (0.022°C/MIN). THUS, ONLY THE INCREASE IN HEATING RATE ATTRIBUTABLE TO ATROPINE WAS PLOTTED. THE CHANGE IN HEATING RATE IS LINEARLY RELATED TO THE DOSE OF ATROPINE BETWEEN 10 AND 1000 ug/kg OF BODY WEIGHT. THE LINES ABOVE AND BELOW THE MEAN REPRESENT THE 95% CONFIDENCE LIMITS FOR GROUPED DATA (N=12 RATS IN EACH DOSE GROUP).

TABLE 1

POTENCY RATIOS OF ANTICHOLINERGIC DRUGS RELATIVE TO ATROPINE

DRUG	DOSE[1] (ug/kg)	HEATING[2] RATE (°C/MIN)	E.A.D.[3] (ug/kg)	POTENCY[4] RATIO
IMIPRAMINE	8000	0.036	28	0.004
AMITRIPTYLINE	3750	0.060	65	0.02
CHLOROPROMAZINE	1000	0.067	84	0.1
ATROPINE	200	0.087	170	1
L-HYOSCYAMINE	100	0.089	182	2
ATROPINE METHYL NITRATE	25	0.067	84	3
SCOPOLAMINE	10	0.079	128	13
SALINE	–	0.022	0	–

[1] ALL DOSES ARE CALCULATED AS FREE BASE

[2] MEAN OF AT LEAST 8 VALUES

[3] EQUIVALENT ATROPINE DOSE (E.A.D.) CALCULATED FROM THE EQUATION $Y=2.83+35.47x$ (Y=LN ATROPINE DOSE AND X=HEATING RATE$-$0.022).

[4] E.A.D./DOSE

DOSES OF OTHER ANTICHOLINERGIC DRUGS WERE CHOSEN TO ELICIT HEATING RATES SIMILAR TO THAT FOR 200 ug/kg OF ATROPINE. TABLE 1 ILLUSTRATES THESE RESULTS. THE EQUIVALENT ATROPINE DOSE (E.A.D.) FOR THE ANTICHOLINERGIC DRUGS WAS DERIVED FROM THE EQUATION OF THE REGRESION LINE FROM FIGURE 2 ($Y=2.83+35.47x$). AGAIN, THE HEATING RATE FOR THE SALINE TREATED RATS (0.022°C/MIN) WAS SUBTRACTED FROM EACH VALUE PRIOR TO ITS USE IN THE EQUATION. THE POTENCY RATIO WAS OBTAINED BY DIVIDING THE EQUIVALENT ATROPINE DOSE BY THE ACTUAL DOSE OF EACH DRUG.

IMPRAMINE (TOFRANIL) AND AMITRIPTYLINE (ELAVIL) ARE BOTH COMMON ANTICHOLINERGIC ANTIDEPRESSANTS. PREVIOUS WORK FROM THIS LAB HAS DEMONSTRATED THAT CHLORPROMAZINE ADMINISTRATION HAS A DETRIMENTAL EFFECT ON THE ABILITY OF RATS TO WORK IN THE HEAT (J. APPL. PHYSIOL. 50, 509, 1981). L-HYOSCYAMINE, THE ACTIVE ISOMER OF ATROPINE, WAS TWICE AS POTENT AS ATROPINE. SINCE THE INHIBITORY EFFECT OF SCOPOLAMINE ON SECRETORY GLANDS IS MORE POTENT THAN THAT OF ATROPINE (GOODMAN AND GILMAN, 1980), THE GREATER POTENCY OF SCOPOLAMINE WAS EXPECTED AND OBSERVED. ATROPINE METHYL NITRATE, THE QUATERNARY AMMONIUM DERIVATIVE OF ATROPINE, MANIFESTED A HIGHER POTENCY RATIO THAN ITS TERTIARY PARENT COMPOUND. THIS OBSERVATION INDICATED THAT THE ANTICHOLINERGIC EFFECT ON HEATING RATE IN THE RAT IS LARGELY A PERIPHERAL PHENOMENON. SINCE THE QUATERNARY COMPOUNDS DO NOT PENETRATE THE BLOOD BRAIN BARRIER TO ANY SIGNIFICANT EXTENT.

TABLE 2

ANTICHOLINESTERASE REVERSAL OF ATROPINE EFFECT

DRUG	DOSE[1] (ug/kg)	DOSE[2] (um)	HEATING[3] RATE (°C/MIN)
NEOSTIGMINE METHYL SULFATE	80	0.24	0.043
PHYSOSTIGMINE SALICYLATE	400	0.96	0.038
PYRIDOSTIGMINE BROMIDE	500	1.90	0.038

[1] DRUG GIVEN 15 MINUTES AFTER 200 ug/kg OF ATROPINE
[2] UM (MICROMOLES) OF FREE BASE
[3] MEAN OF AT LEAST 6 ANIMALS

SINCE ATROPINE RESULTED IN AN INCREASE IN THE RATE OF HEATING BY BLOCKING SALIVATION, A REVERSAL OF ITS EFFECT IS INDICATED BY A DECREASE IN HEATING RATE TOWARD SALINE CONTROL VALUES. THE RESULTS IN TABLE 2 DEMONSTRATE THAT THE ADMINISTRATION OF 400 ug/kg OF PHYSOSTIGMINE OR 500 ug/kg OF PYRIDOSTIGMINE OR 80 ug/kg OF NEOSTIGMINE REDUCED THE HEATING RATE OF A 200 ug/kg DOSE OF ATROPINE (0.087°C/MIN) BY 75%.* (THERE IS NO SIGNIFICANT DIFFERENCE BETWEEN THE HEATING RATE FOR NEOSTIGMINE (0.043±0.008°C/MIN) AND THE RATES FOR THE OTHER 2 CARBAMATES.) IF THE QUANTITIES OF THE DRUGS ARE CONVERTED TO MICROMOLES (UM) OF THE FREE BASE, IT APPEARS THAT 8 TIMES AS MUCH PYRIDOSTIGMINE OR 4 TIMES AS MUCH PHYSOSTIGMINE IS NEEDED TO ACHIEVE THE SAME CHANGE IN HEATING RATE AS IS SEEN WITH 0.24 um/kg OF NEOSTIGMINE. (CONVERSION TO UM WAS UNNECESSARY IN THE ANTICHOLERGIC POTENCY STUDY BECAUSE ALL THE DRUGS USED ARE OF A VERY SIMILAR MOLECULAR WEIGHT.) PLASMA CHOLINESTERASE LEVELS WERE MEASURED IN RATS GIVEN THESE CARBAMATES WITHOUT HEAT-STRESSING THE ANIMALS. THE FOLLOWING % INHIBITIONS WERE SEEN AT 60 MINUTES AFTER THE INJECTION: NEOSTIGMINE 80 ug/kg - 38%, PHYSOSTIGMINE 200 ug/kg (400 ug/kg WITHOUT ATROPINE WAS FATAL) - 35%, PYRIDOSTIGMINE 500 ug/kg -54%, AND ATROPINE 200 ug/kg PLUS PYRIDOSTIGMINE 500 ug/kg - 53%.

* The increase in heating rate from .022 for saline to .087°C/min for 200 ug/kg of atropine is .065°C/min. It is this increase that is reduced by 75% since the increase for atropine plus a carbamate is .038 - .022 = .016 °C/min.

CONCLUSION

USING THE HEAT-STRESSED RAT, WE HAVE DETERMINED THAT ATROPINE INCREASES THE HEATING RATE FOR UP TO 3 HOURS AFTER ADMINISTRATION, AND THAT THIS INCREASE IS DOSE-DEPENDENT OVER THE RANGE OF 10-1000 ug/kg. THIS DOSE-DEPENDENT ELEVATION IN HEATING RATE HAS BEEN USED TO ASSESS THE POTENCY OF OTHER ANTICHOLINERGIC DRUGS. ADDITIONALLY, THE EFFECT OF ATROPINE ON HEATING RATE IS ATTENUATED BY AT LEAST ONE CLASS OF ANTICHOLINESTERASES, THE CARBAMATES. THEREFORE, THIS MODEL IS A PROMISING TOOL WITH WHICH TO EVALUATE OTHER ANTICHOLINERGIC AND ANTICHOLINESTERASE COMPOUNDS.

BEHAVIORAL RHYTHMOMETRY IN RATS: TESTING LONG-TERM EFFICACY OF TREATMENTS FOR ORGANOPHOSPHATE POISONING

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Behavioral circadian rhythms provide a sensitive baseline for the detection of chronic effects of organophosphate (OP) poisoning. Diisopropyl Phosphorofluoridate (DFP) and other OP poisons have been reported to disrupt normal sleep and electroencephalogram patterns in humans and rhesus monkeys for periods exceeding one year. Recently, our lab has demonstrated that DFP chronically disrupts normal circadian patterns of eating, running, and drinking in rats. Although the identification of antidotes and prophylactic agents to enhance survival following OP poisoning is obviously of critical importance, there is now growing evidence that such survivors may be permanently brain-damaged. Thus, it is equally important to identify substances which also protect against chronic effects of OP poisons. Since behavioral rhythms are chronically sensitive to the effects of OP poisons, behavioral rhythmometry may provide a method for the detection of those survival enhancing agents which also protect against chronic effects. The present research compared the behavioral rhythms of normal and DFP treated rats with the rhythms of rats which had been pre-treated with one of a variety of substances regularly used in the treatment of OP poisoning and then exposed to DFP. A substance would be identified as being potentially useful in ameliorating the chronic effects of DFP if that treatment resulted in behavioral rhythms which were either normal or less disrupted than those of animals receiving DFP alone.

Adult male Sprague-Dawley rats were injected with vehicle (saline or peanut oil), 1.0 mg/kg DFP, 2.6 mg/kg DFP, or 2.6 mg/kg DFP with pre-treatment. Pre-treatments included 25.0 mg/kg atropine sulfate, 12.5 mg/kg 2-PAM chloride, or 4.0 mg/kg diazepam. Two days post-injection twelve rats were housed in hanging wire home cages equipped with devices which dispensed 45 mg food pellets when operated by the rat. PDP-8 computers monitored the devices to provide a record of the time of day during which feeding occurred. On day 17 post-injection, six rats were removed from the feeding cages and placed in computer-monitored running wheels for an additional 12 days. Standard laboratory environmental conditions were maintained including a 12/12 light dark cycle with light onset at 0600 hours.

Consistent with past results, both low and high doses of DFP produced a marked disruption of normal feeding patterns relative to the vehicle controls. No pre-treatment group showed a less disrupted feeding pattern than the comparable DFP treated group during the first 16 days of the experiment. All treatment groups showed an increase in rhythmicity during the first 16 days which was not different from that of the DFP groups. During days 24 to 30, running patterns remained disrupted relative to controls in the 2.6 mg/kg DFP and 2-PAM with DFP groups. However, the 1.0 mg/kg DFP group was indistinguishable from controls, and the diazepam pre-treated group showed signs of increased rhythmicity relative to the 2.6 mg/kg DFP group. Traditional measures of treatment effectiveness, such as body temperature, weight, and lethality, were also observed. Rhythmic disruptions persisted well beyond a return to normal body weight and temperature, and were not related to lethality. The gradations in both the initial degree of rhythmicity and the rate of recovery of rhythmicity seen in these groups indicate that behavioral rhythmometrics can be an important tool for the identification of substances which are efficacious in the treatment of the chronic effects of OP poisoning.

□ CONTROL

○ 1.0 mg/kg DFP

● 2.6 mg/kg DFP

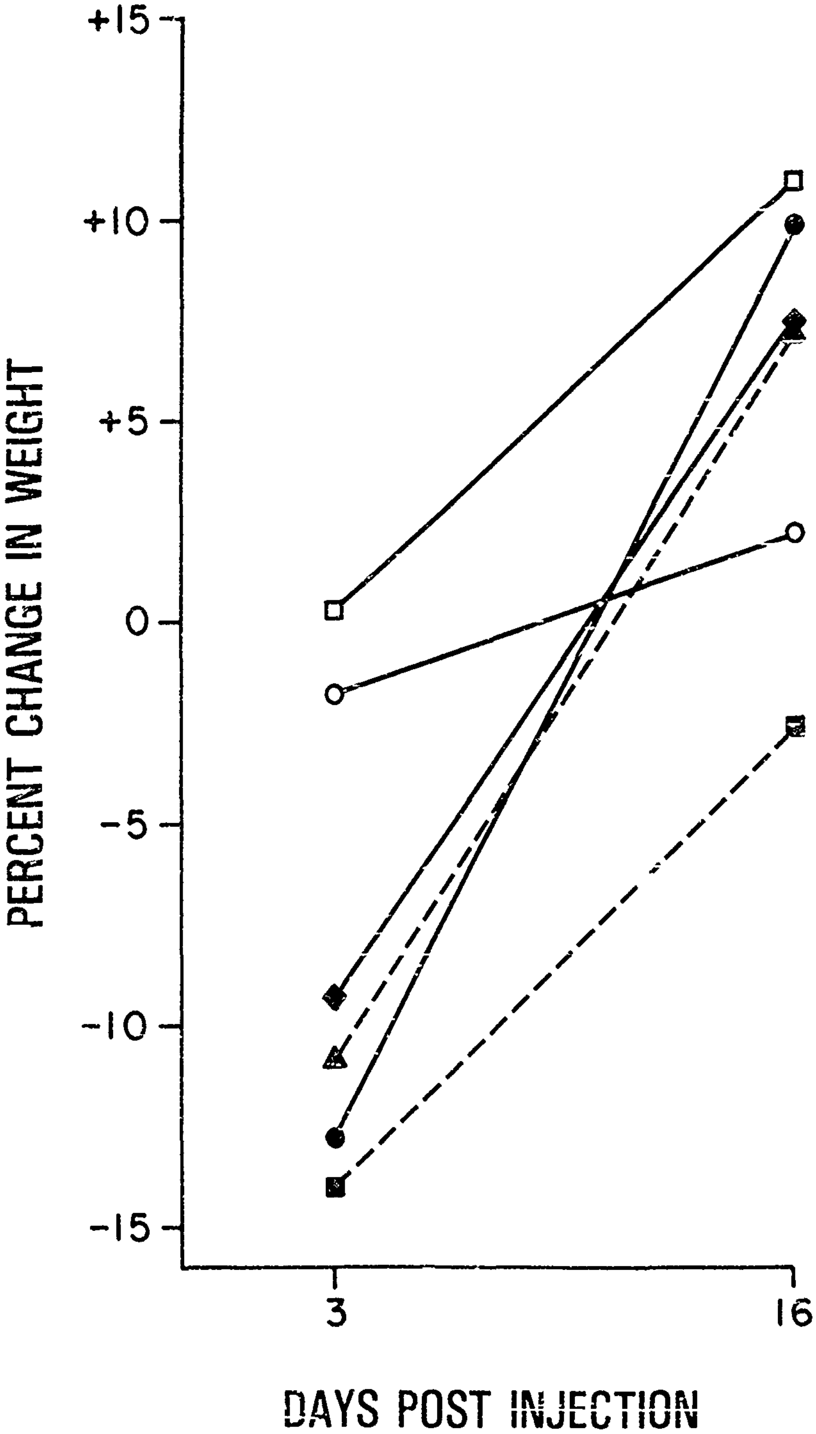
▲ 2.6 DFP + 2–PAM

◆ 2.6 DFP + DIAZEPAM

■ 2.6 DFP + ATROPINE SULPHATE

△ 2.6 DFP + 2–PAM + ATROPINE

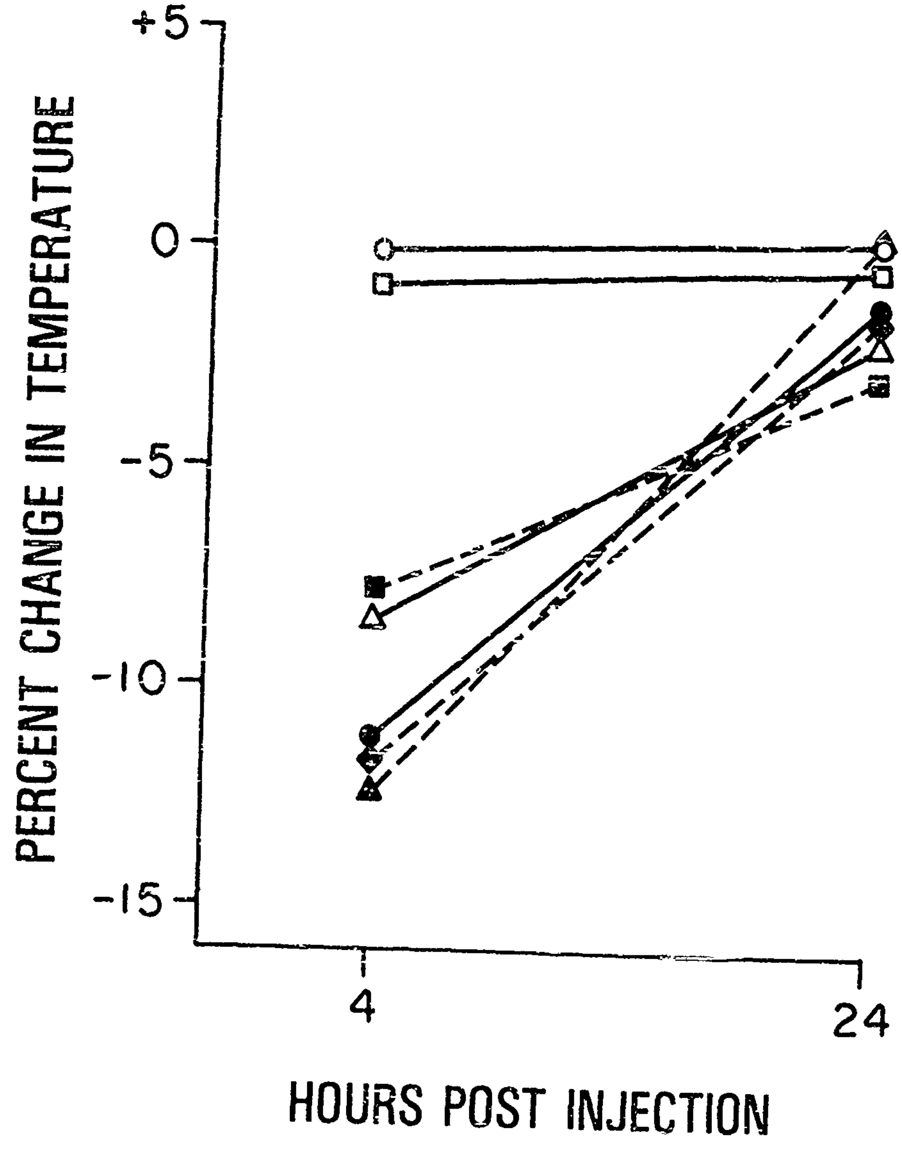
* $p < .05$ RELATIVE TO CONTROL



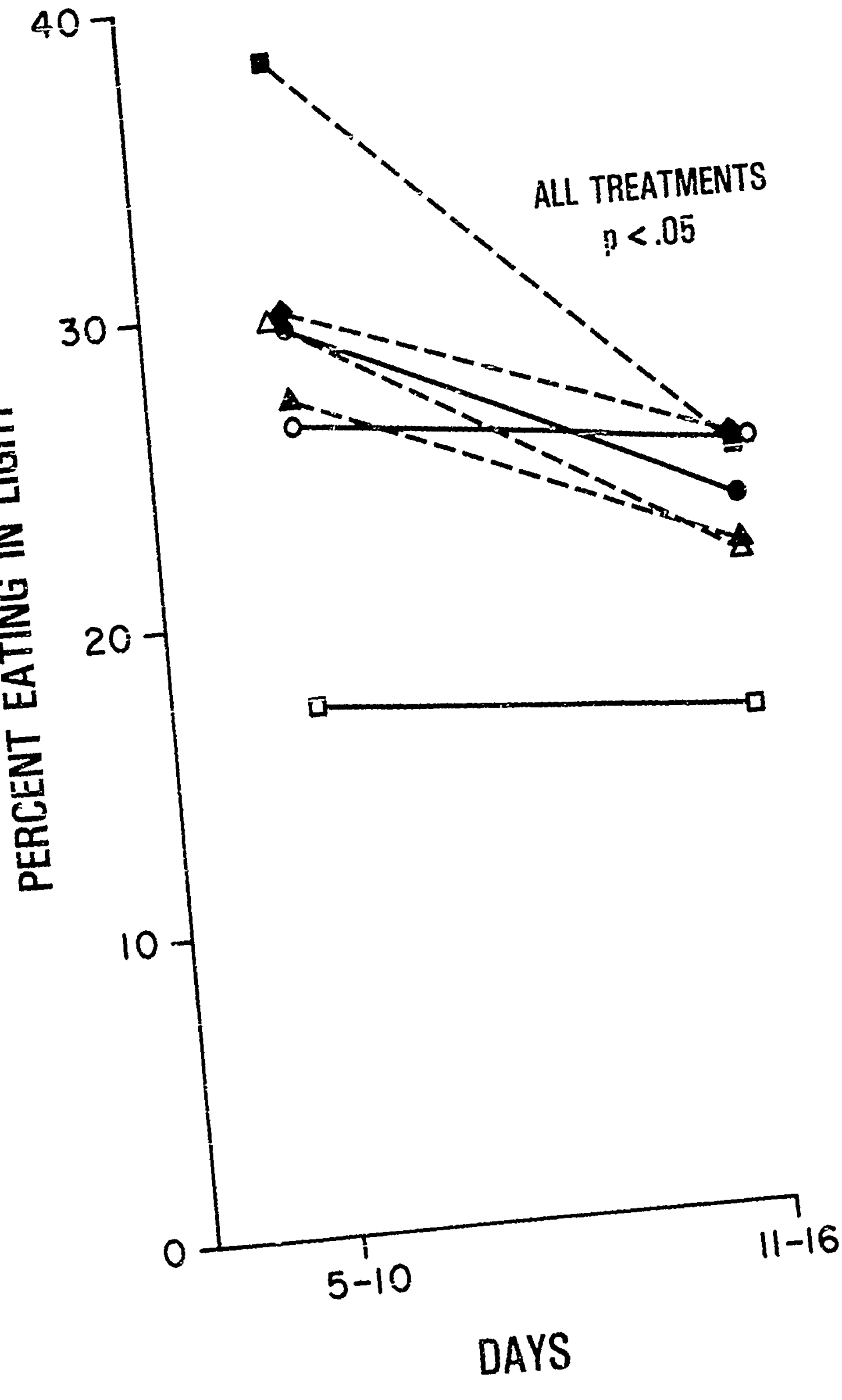
+15
+10
+5
0
-5
-10
-15
PERCENT CHANGE IN WEIGHT
3
16
DAYS POST INJECTION

A-1347

SHORT-TERM CHANGES IN BODY TEMPERATURE

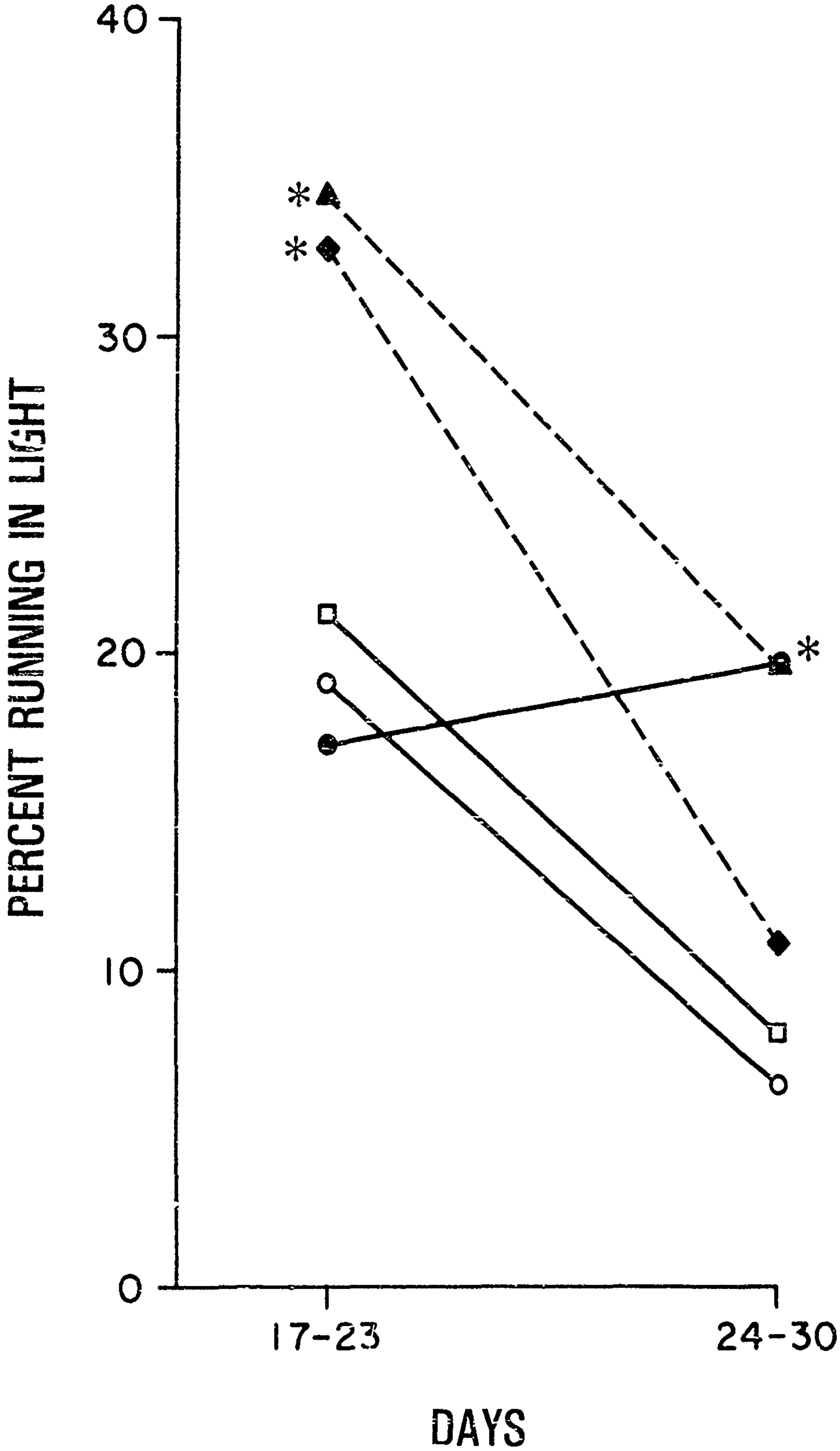


A-1348



ALL TREATMENTS
p < .05
PERCENT EATING IN LIGHT
40
30
20
10
0
5-10
11-16
DAYS

A-1349



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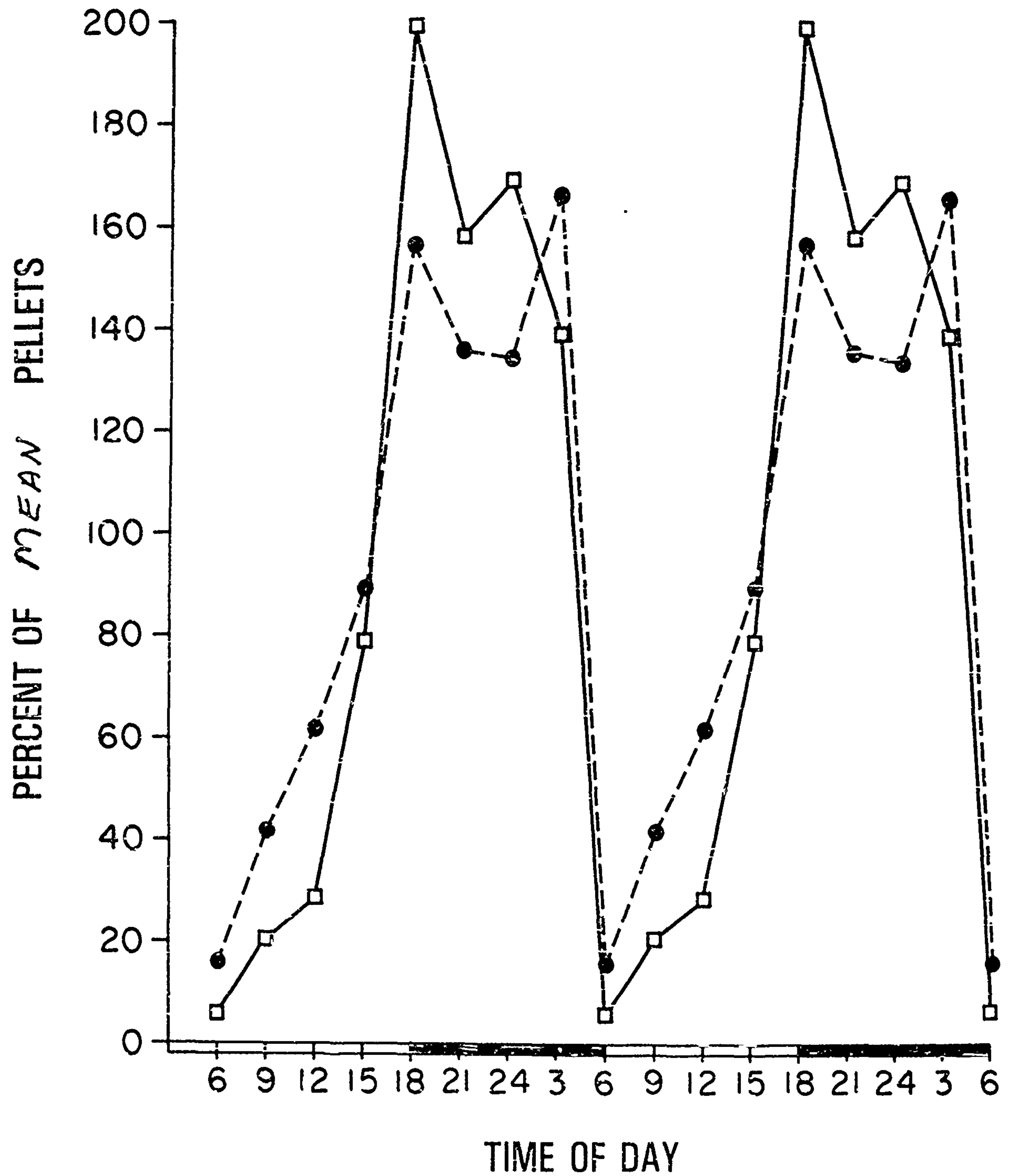
CIRCADIAN DISTRIBUTION OF EATING DAYS 5–16



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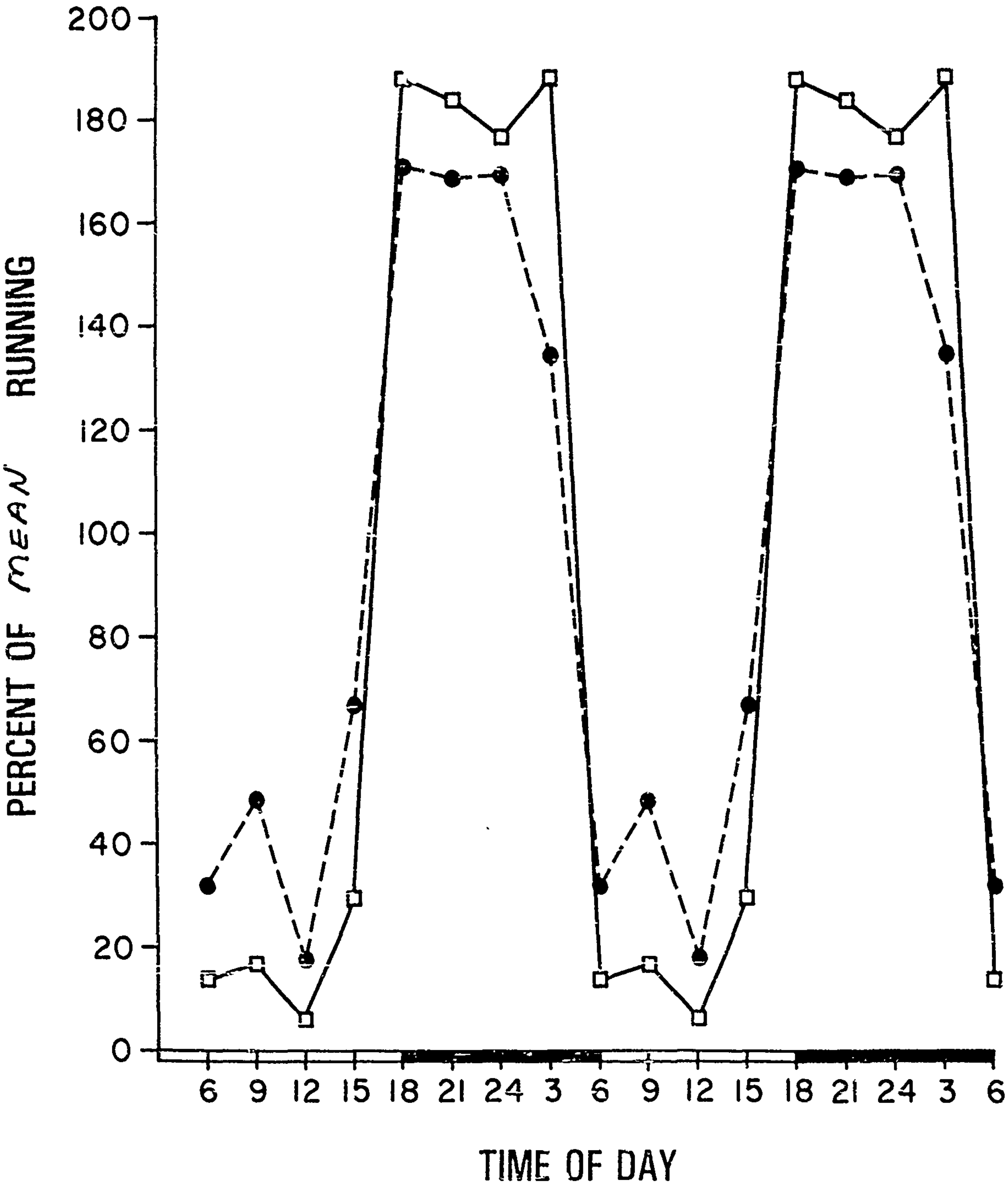
CIRCADIAN DISTRIBUTION OF RUNNING DAYS 17–30



A-1352

CHOLINESTERASE INHIBITORS: ANIMAL MODELS OF BEHAVIORAL EFFECTS AND THERAPEUTIC INTERVENTIONS

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INTRODUCTION

MANIPULATION OF THE CHOLINERGIC SYSTEM IN RATS CAN AFFECT BEHAVIOR IN A VARIETY OF TASKS. THE FOLLOWING EXPERIMENTS EXAMINE THE LONG-TERM EFFECTS OF THE ORGANOPHOSPHATE CHOLINESTERASE INHIBITOR, SOMAN. WHEN GIVEN IN HIGH DOSES, THIS AGENT ACTS AS AN EXCITOTOXIN AND CAN CAUSE PERMANENT DAMAGE TO THE CENTRAL NERVOUS SYSTEM. THE STUDIES DESCRIBED BELOW WERE PERFORMED IN ORDER TO ASSESS THE PERFORMANCE OF SOMAN-TREATED RATS IN SEVERAL BEHAVIORAL TESTS.

METHOD

SUBJECTS. MALE, ALBINO RATS, WEIGHING 400-500 G AT THE BEGINNING OF TESTING, WERE USED. APPROXIMATELY ONE MONTH BEFORE THE ONSET OF BEHAVIORAL TESTING, ALL RATS WERE INJECTED WITH EITHER 85UG OF SOMAN, 50UG OF SOMAN OR PHYSIOLOGICAL SALINE.

PROCEDURES.

UNLESS OTHERWISE SPECIFIED, ALL RATS WERE HANDLED AND FOOD-DEPRIVED FOR 2 DAYS PRIOR TO BEING TESTED IN THE FOLLOWING TASKS:

1) LOCOMOTOR ACTIVITY IN AN OPEN FIELD

ON TWO CONSECUTIVE DAYS, 4 RATS FROM EACH OF THE GROUPS (HIGH DOSE, LOW DOSE, AND SALINE) WERE PLACED INDIVIDUALLY IN A PLYWOOD BOX WHICH MEASURED 24 SQ. INCHES. THE WALLS WERE 18 INCHES HIGH, AND THE FLOOR OF THE BOX WAS PAINTED WITH A GRID PATTERN OF 9 EQUALLY SIZED BOXES. EACH RAT WAS ALLOWED TO EXPLORE FOR 10 MINUTES, DURING WHICH THE NUMBER OF BOXES ENTERED WAS RECORDED.

2) REACTIVITY TO TACTILE STIMULATION

THE SAME RATS USED IN THE ACTIVITY TEST WERE USED AGAIN IN THIS PROCEDURE. THEIR REACTIVITY WAS RATED ON A SCALE FROM Ø-3 UPON PRESENTATION OF EACH OF THE FOLLOWING STIMULI: A) A PUFF OF AIR ON THE RAT'S BACK B) A SMALL STICK TOUCHING THE RAT'S BACK C) A SMALL STICK TOUCHING THE RAT'S NOSE D) A GLOVED HAND PICKING UP THE RAT. THUS, THE MAXIMUM POSSIBLE REACTIVITY SCORE WAS 12.

3) REVERSAL OF A POSITION HABIT ON A T MAZE

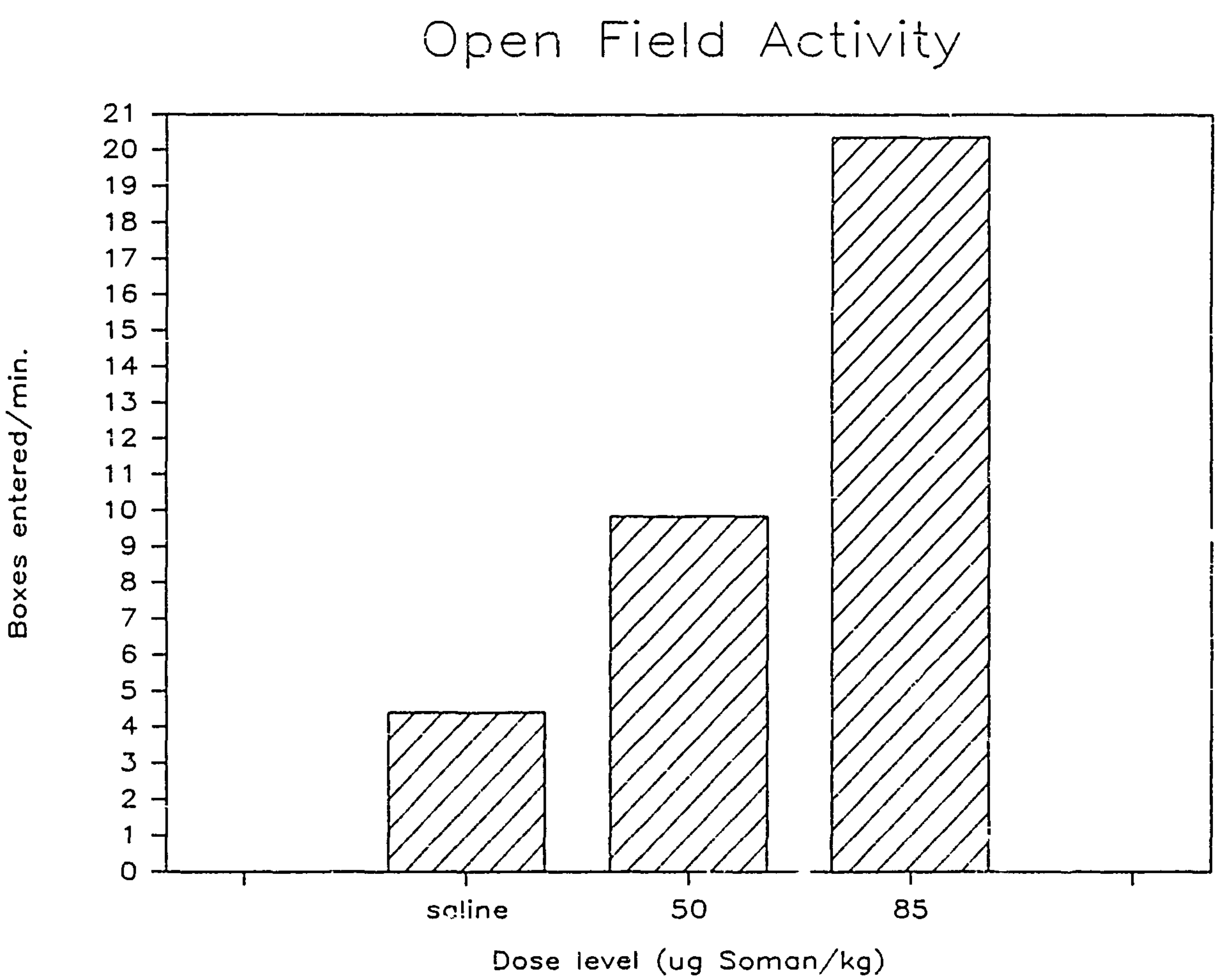
TWO RATS FROM EACH GROUP WERE TRAINED TO FIND FOOD IN EITHER THE LEFT OR RIGHT ARM OF AN ELEVATED T MAZE. AFTER A RAT HAD REACHED A CRITERION OF PERFORMANCE, THE LOCATION OF FOOD ON THE MAZE WAS REVERSED. THIS PROCEDURE WAS REPEATED FOR A MAXIMUM OF 5 REVERSALS.

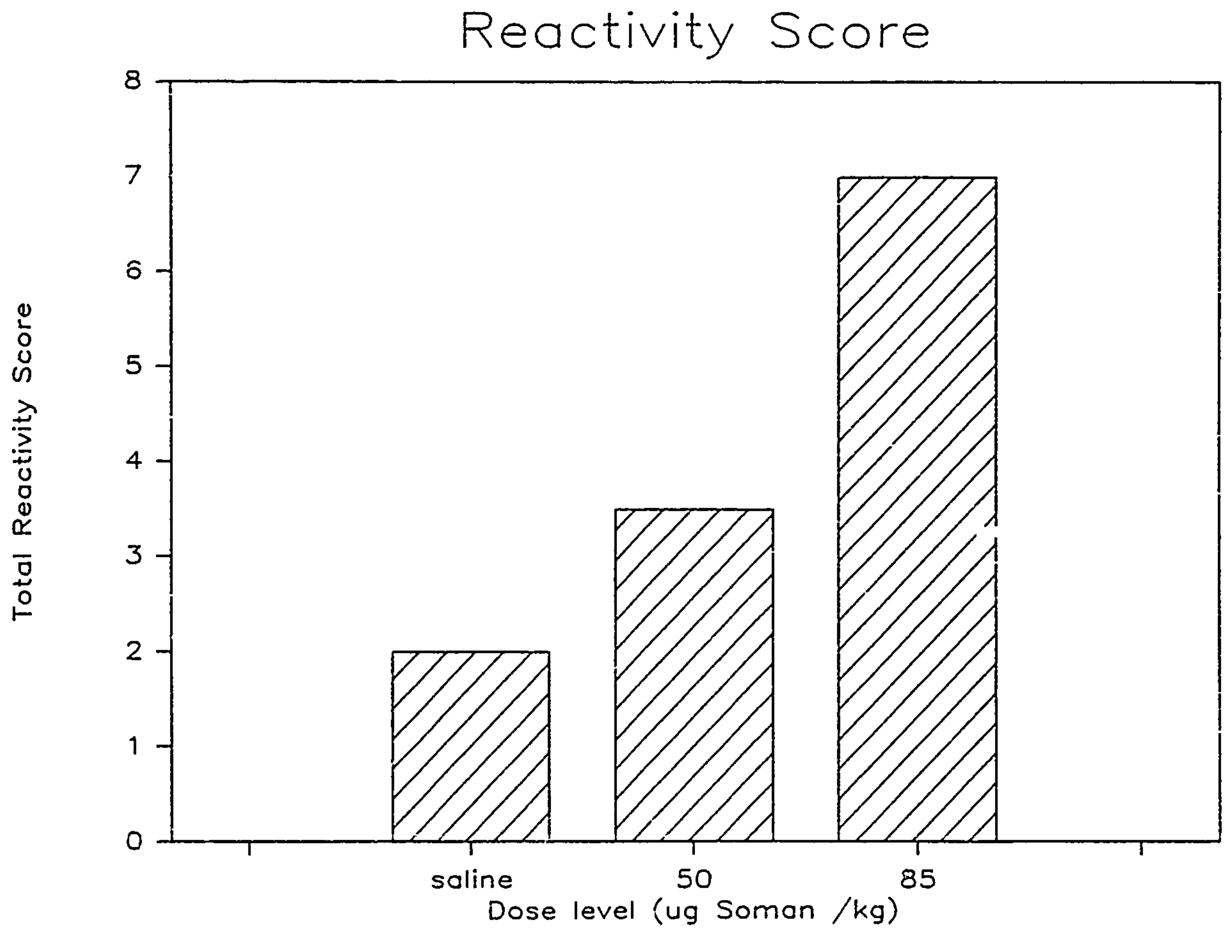
4) WORKING MEMORY AND DRUG THERAPY

EIGHT RATS FROM THE GROUP THAT HAD RECEIVED A HIGH DOSE OF SOMAN WERE FOOD DEPRIVED TO 85% OF THEIR AD LIB. WEIGHTS. ALL RATS WERE TRAINED TO FIND FOOD ON A TWELVE ARM RADIAL MAZE. THE FOOD WELL AT THE END OF EACH ARM WAS BAITED WITHOUT REPLACEMENT. THUS, THE OPTIMAL FORAGING STRATEGY FOR A RAT WAS TO VISIT EACH ARM ONLY ONCE. IN ORDER TO DO THIS RAT HAD TO REMEMBER WHERE HE HAD BEEN WITHIN A GIVEN TRIAL AND MAKE SUBSEQUENT CHOICES ACCORDINGLY. THEREFORE, AN ERROR WAS DEFINED AS A RETURN VISIT TO AN ARM FROM WHICH THE FOOD HAD PREVIOUSLY BEEN TAKEN.

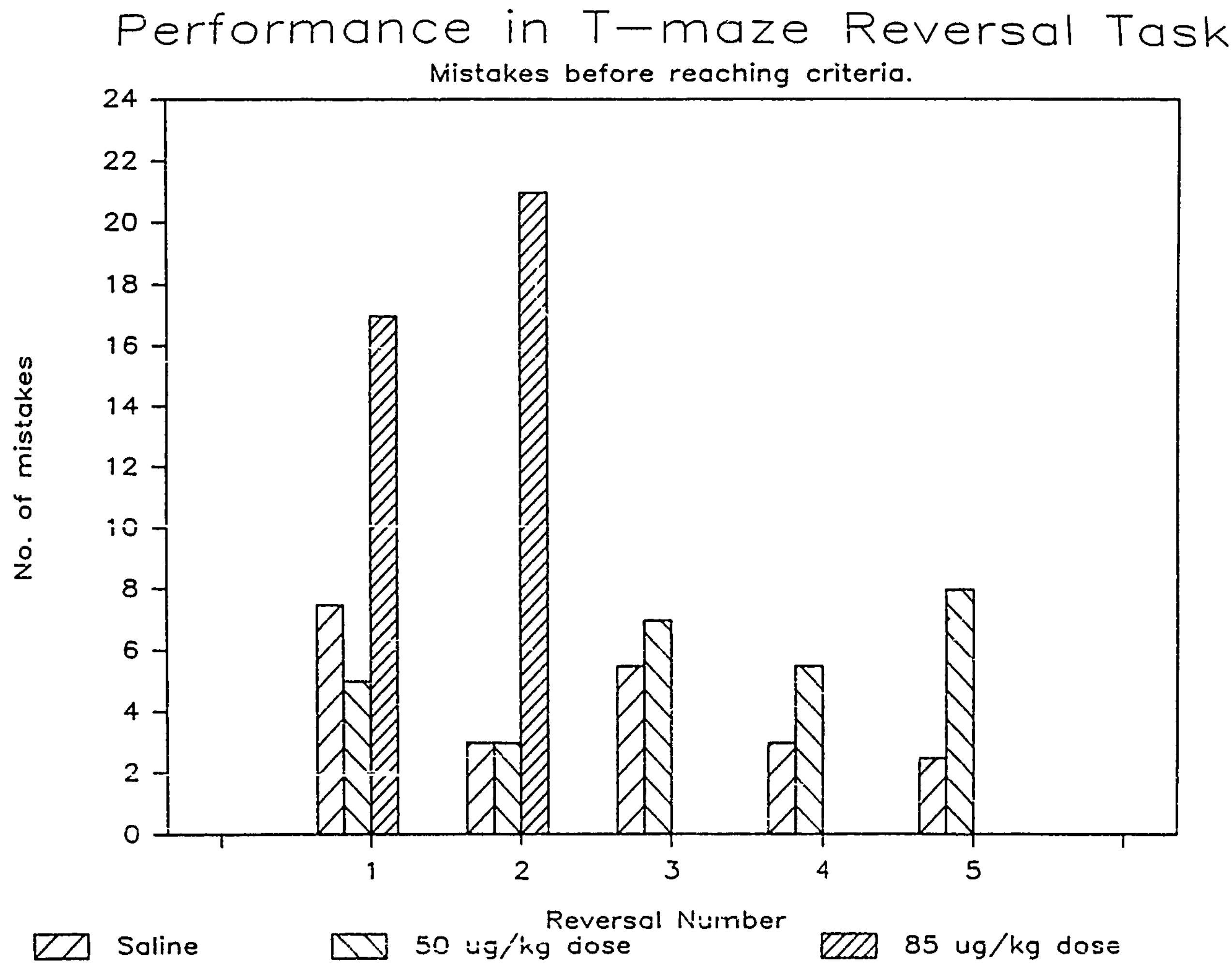
FOUR RATS WERE GIVEN INTRAPERITONEAL INJECTIONS OF PENTOXIFYLLINE (20 MG/KG) AND FOUR WERE INJECTED WITH PHOSPHATE-BUFFERED SALINE WITHIN ONE HALF HOUR OF THE BEGINNING OF THE DAY'S TESTING. THEY WERE GIVEN THREE TRIALS DAILY WITH AN INTERTRIAL INTERVAL OF AT LEAST ONE HOUR FOR A GIVEN RAT. A TRIAL WAS TERMINATED EITHER AFTER THE RAT HAD BEEN TO ALL TWELVE ARMS OF THE MAZE, OR HAD MADE A TOTAL OF 18 CHOICES, OR 10 MINUTES HAD PASSED.

FOR THE SAKE OF COMPARISON, THE FIGURE BELOW ALSO SHOWS THE RADIAL MAZE PERFORMANCE OF TWO GROUPS OF RATS THAT WERE RUN IN A SEPARATE EXPERIMENT AND WERE NOT TREATED WITH SOMAN. ONE GROUP HAD BILATERAL, IBOTENIC ACID LESIONS OF THE BASAL FOREBRAIN CHOLINERGIC SYSTEM. RATS IN THE THE OTHER GROUP WERE SHAM LESION CONTROLS.





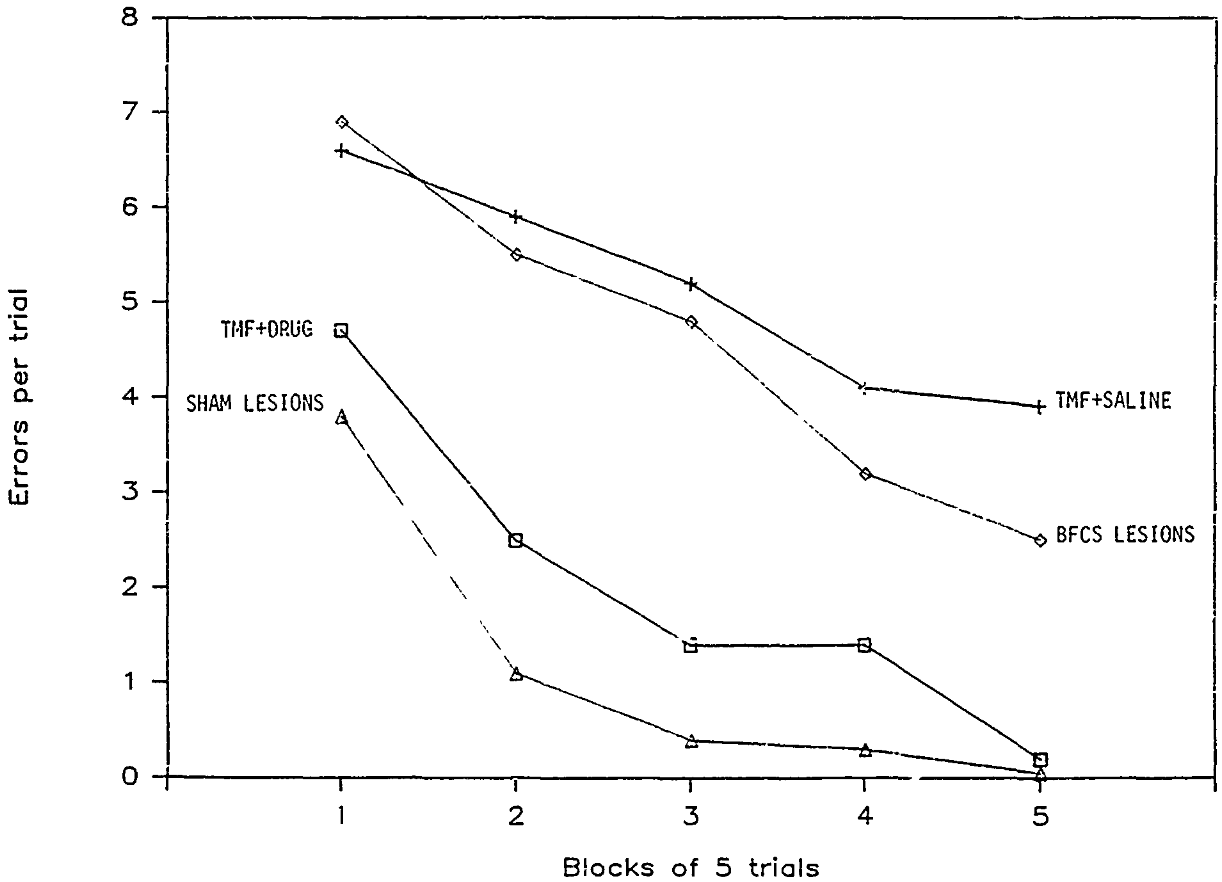
Reactivity Score
Total Reactivity Score
0
1
2
3
4
5
6
7
8
saline
50
85
Dose level (ug Soman /kg)



Performance in T-maze Reversal Task
Mistakes before reaching criteria.
No. of mistakes
0
2
4
6
8
10
12
14
16
18
20
22
24
1
2
3
4
5
Reversal Number
Saline
50 ug/kg dose
85 ug/kg dose

A-1356

Choice accuracy on radial maze



RATIONALE FOR DRUG TREATMENT

THE TREATMENT WAS CHOSEN TO AUGMENT ACTIVITY IN CHOLINERGIC NEURONS THAT SURVIVED THE LESION.

AGENT	DOSE	PRESUMED ACTION
PENTOXIFYLLINE	20 MG/KG I.P. THRICE WEEKLY	INCREASES OXIDATIVE METABOLISM LEVELS OF INTRA-CELLULAR ATP, AND O_2 CONSUMPTION

CONCLUSIONS

RATS THAT WERE TREATED WITH SOMAN TENDED TO BE HYPERACTIVE, HYPERREACTIVE, AND IMPAIRED IN BOTH REVERSAL LEARNING AND WORKING MEMORY PERFORMANCE. MOREOVER, THESE DATA DEMONSTRATE THAT THERE WAS A DOSE-RESPONSE CURVE FOR THE EFFECTS OF SOMAN IN THESE BEHAVIORAL TESTS. THESE FINDINGS ARE CONSISTENT WITH THOSE FROM STUDIES OF LESIONS AND PHARMACOLOGICAL BLOCKADE OF THE CHOLINERGIC SYSTEM. IN ADDITION, THE ADMINISTRATION OF PENTOXIFYLLINE MAY IMPROVE THE PERFORMANCE OF SOMAN-TREATED RATS ON THE RADIAL MAZE.

MORE WORK MUST BE DONE IN ORDER TO VERIFY THE BEHAVIORAL EFFECTS OF SOMAN. FIRST, ADDITIONAL RATS MUST BE TESTED IN THE PARADIGMS DESCRIBED ABOVE IN ORDER TO DETERMINE WHETHER THE PROMISING TRENDS SHOWN HERE WILL BE STATISTICALLY SIGNIFICANT EFFECTS. OTHER TESTS SHOULD ALSO BE DONE IN ORDER TO GET A MORE COMPLETE BEHAVIORAL PROFILE OF SOMAN-TREATED RATS. THESE FINDINGS, COMBINED WITH DETAILED HISTOLOGICAL AND NEUROCHEMICAL EVALUATIONS, WILL PROVIDE A BROAD PERSPECTIVE ON THE NEURAL AND BEHAVIORAL CONSEQUENCES OF SOMAN EXPOSURE. ONCE THESE CHARACTERISTICS ARE DEFINED, A SYSTEMATIC APPROACH TO THERAPEUTIC INTERVENTION CAN BE IMPLEMENTED.

CORRELATION BETWEEN PHARMACOLOGICAL EFFECTS AND CHOLINERGIC SYSTEMS IN MICE AFTER DIISOPROPYLFLUOROPHOSPHATE ADMINISTRATION

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ABSTRACT

STUDIES WERE CARRIED OUT TO INVESTIGATE A POSSIBLE CORRELATION BETWEEN ACETYLCHOLINESTERASE (ACHE) INHIBITION, OTHER CHANGES IN BRAIN CHOLINERGIC FUNCTION AND PHARMACOLOGICAL EFFECTS OF DFP IN MALE ICR MICE. A DOSE RELATED (0.5, 1, 2 MG/KG, I.V.) HYPOTHERMIA OCCURRED WITHIN 30 MIN AND LASTED FOR 4 HR. SPONTANEOUS ACTIVITY DECREASED IN A DOSE RELATED FASHION WITHIN 5 MIN AND UPTO 30 MIN. DFP (2 MG/KG) INHIBITED ACHE ACTIVITY IN WHOLE BRAIN IMMEDIATELY AND THIS INHIBITION LASTED UP TO 24 HR. BRAIN ACHE ACTIVITY WAS MEASURED IN WHOLE BRAIN AND IN VARIOUS BRAIN AREAS 10 MIN AFTER I.V. INJECTION OF DFP (0.5 - 2.0 MG/KG). A DOSE RELATED DECREASE WAS OBSERVED IN WHOLE BRAIN AS WELL AS IN ALL BRAIN AREAS. EFFECT OF DFP (0.5 - 2 MG/KG) WAS ALSO STUDIED ON LEVELS OF ACETYLCHOLINE (ACH), CHOLINE (CH) AND ON TURNOVER RATE OF ACH IN WHOLE BRAIN AND IN BRAIN AREAS. ACH LEVELS WERE SIGNIFICANTLY INCREASED IN WHOLE BRAIN, MIDBRAIN AND HIPPOCAMPUS BUT CH LEVELS AND ACH TURNOVER RATES WERE NOT EFFECTED. THE EFFECT OF DFP TREATMENT ON BRAIN ACHE INHIBITION WAS NOT CORRELATED TEMPORALLY WITH DECREASES IN SPONTANEOUS ACTIVITY OR BODY TEMPERATURE. DOSES OF DFP WHICH INHIBITED ACHE AND PRODUCED PHARMACOLOGICAL CHANGES INCREASED ACH LEVELS BUT DID NOT ALTER ACH TURNOVER. (SUPPORTED BY U.S.A.M.R.D.C. CONTRACT 17-82-C-2174).

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INTRODUCTION

DIISOPROPYLFLUROPHOSPHATE (DFP) IS A POTENT AND IRREVERSIBLE ORGANOPHOSPHATE ACETYLCHOLINESTERASE (ACHE) INHIBITOR PRODUCING PRONOUNCED PERIPHERAL AND CENTRAL CHOLINERGIC STIMULATION. ENHANCED CHOLINERGIC ACTIVITY CONSEQUENT TO ACHE INHIBITION HAS TRADITIONALLY BEEN BELIEVED TO BE THE CAUSE OF THE MANY TOXICOLOGICAL, BEHAVIORAL, NEUROLOGICAL AND BIOCHEMICAL EFFECTS OF ORGANOPHOSPHATES.

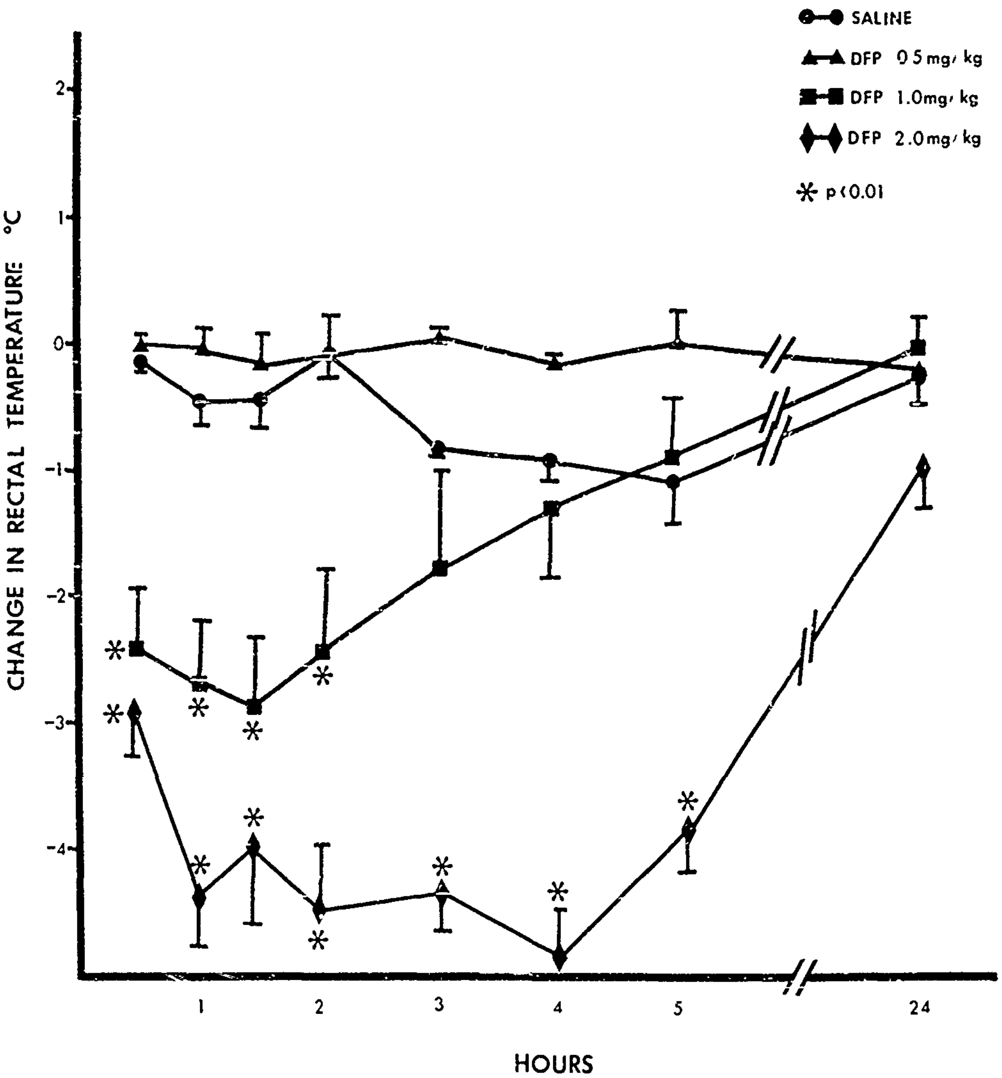
THE ACUTE EFFECTS OF DFP ON ACHE ACTIVITY AND ON ANIMAL BEHAVIOR HAVE BEEN STUDIED IN VARIOUS SPECIES AND IN DIFFERENT EXPERIMENTAL CONDITIONS. A POSSIBLE CORRELATION BETWEEN ACHE INHIBITION AND CHANGES IN OTHER CHOLINERGIC PARAMETERS OR IN BEHAVIORAL EFFECTS HAS BEEN UNDERTAKEN RARELY. THE PRESENT STUDY WAS DESIGNED TO ELUCIDATE CHANGES IN CHOLINERGIC FUNCTION IN WHOLE MOUSE BRAIN AND IN DISCRETE BRAIN AREAS FOLLOWING ACUTE ADMINISTRATION OF DFP AND TO DETERMINE WHETHER THESE CHANGES CORRELATE WITH ITS PHARMACOLOGICAL EFFECTS. THE PARAMETERS OF CHOLINERGIC ACTIVITY INCLUDE ACHE ACTIVITY, ENDOGENOUS LEVELS OF ACETYLCHOLINE AND CHOLINE AND TURNOVER RATE OF ACH. ALTERATIONS IN BODY TEMPERATURE AND SPONTANEOUS ACTIVITY WERE THE PHARMACOLOGICAL PARAMETERS MONITORED.

EFFECT OF DFP (I.V.) ON ACETYLCHOLINESTERASE ACTIVITY IN MOUSE BRAIN REGIONS[A]

	% INHIBITION		
	1 MG/KG	1.5 MG/KG	2.0 MG/KG
MEDULLA-PONS	80 ± 5	93 ± 4	92 ± 0.0
CEREBELLUM	67 ± 16	94 ± 6	88 ± 5
MIDBRAIN	78 ± 0.0	90 ± 2	95 ± 2
CORPUS STRIATUM	73 ± 3	83 ± 3	93 ± 1
HIPPOCAMPUS	82 ± 0	91 ± 2	92 ± 1
CORTEX	74 ± 4	86 ± 3	91 ± 2

[A] MICE WERE SACRIFICED 10 MIN AFTER DFP ADMINISTRATION.

TIME COURSE OF DFP INDUCED HYPOTHERMIA IN MICE



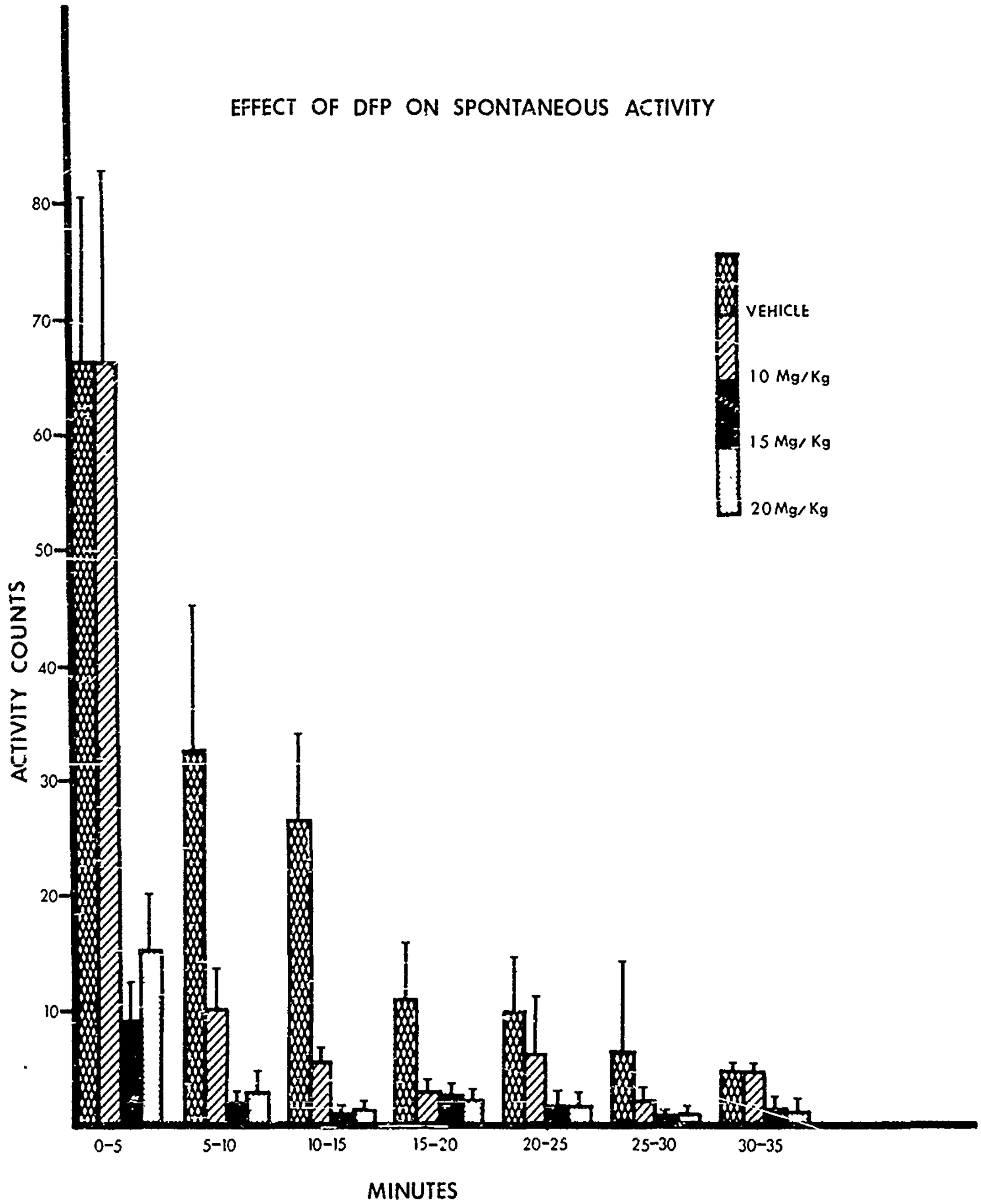
THE EFFECT OF VARIOUS DOSES OF DFP ON CHOLINERGIC FUNCTION IN MOUSE BRAIN

	ACH NMOL/G	CHOLINE NMOL/G	T.O. RATE OF ACH NMOL/G/MIN
SALINE	23.97 ± 1.06	48.12 ± 4.6	14.2 ± 1.3
0.5 MG/KG	24.37 ± 0.81	46.7 ± 3.2	15.5 ± 2.7
1.0 MG/KG	26.16 ± 1.45	53.6 ± 5.8	16.8 ± 4.0
2.0 MG/KG	34.07 ± 1.03*	56.5 ± 5.1	14.2 ± 2.1

MICE WERE SACRIFICED 10 MIN AFTER DFP ADMINISTRATION.

THE FIGURES ARE MEANS ± S.E.

* P < .01


EFFECT OF DFP ON SPONTANEOUS ACTIVITY
ACTIVITY COUNTS
80
70
60
50
40
30
20
10
VEHICLE
10 Mg/Kg
15 Mg/Kg
20 Mg/Kg
0-5
5-10
10-15
15-20
20-25
25-30
30-35
MINUTES

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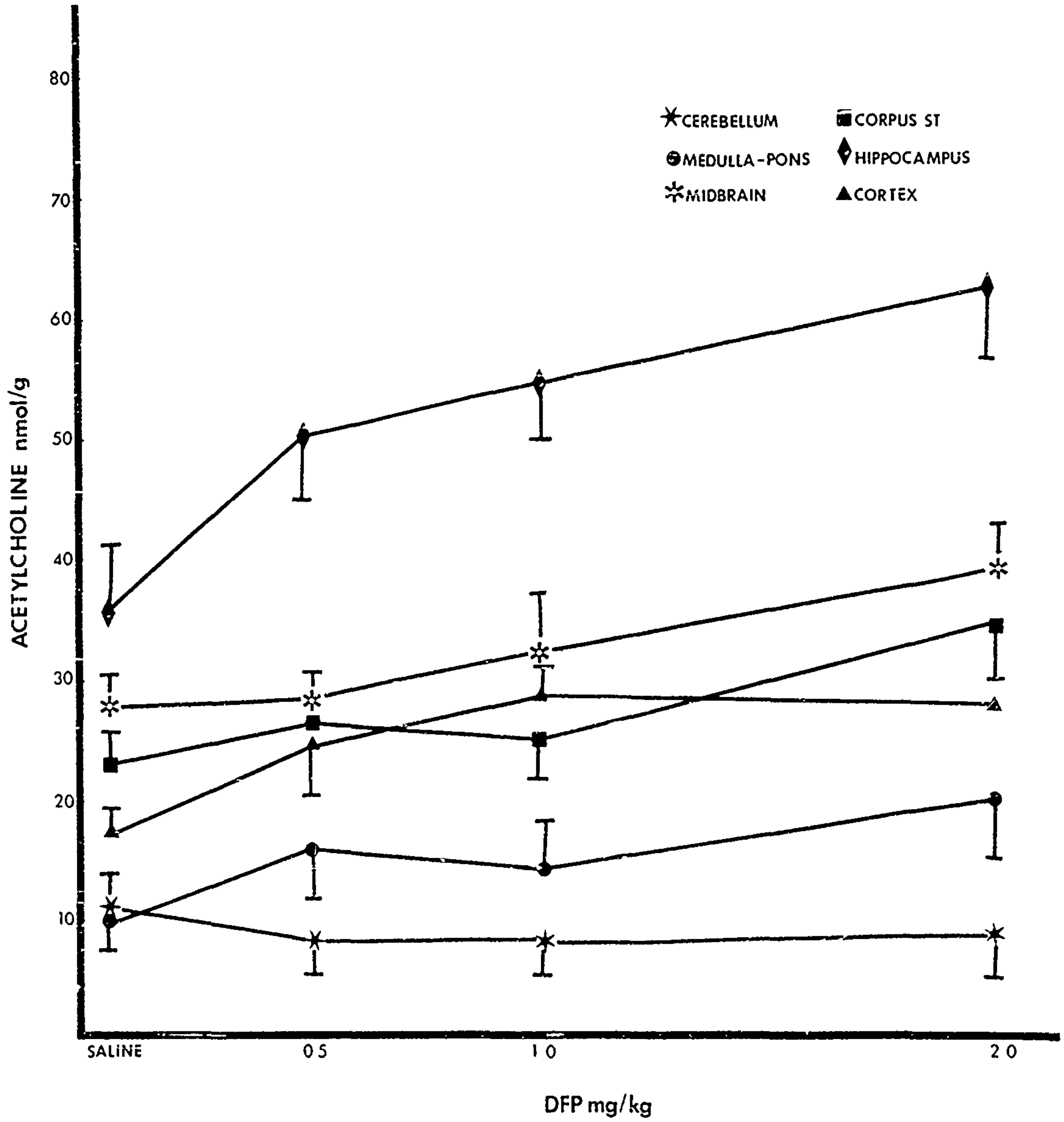
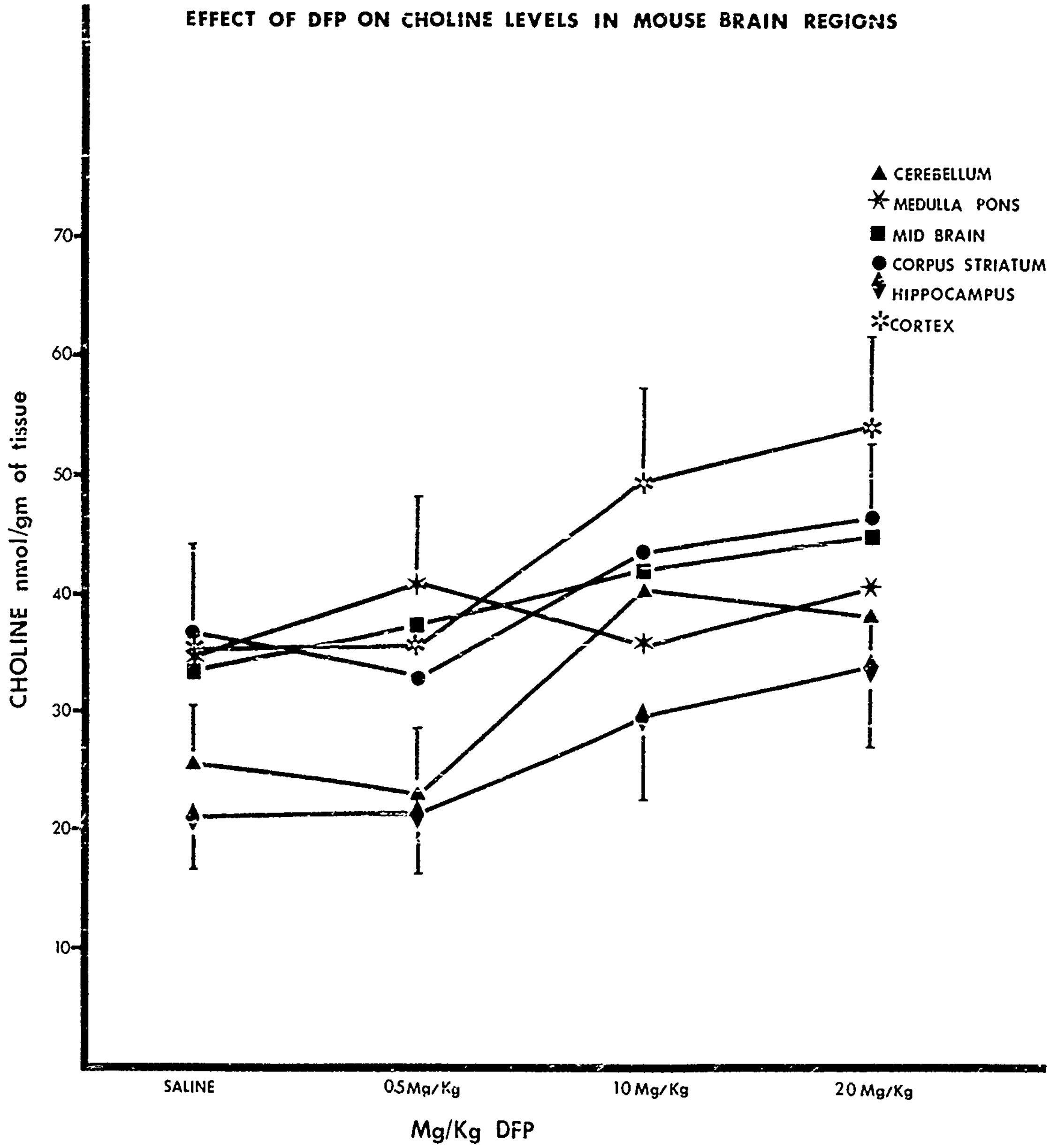
EFFECTS OF DFP ON ACETYLCHOLINE LEVELS IN MOUSE BRAIN REGIONS



EFFECT OF DFP (2 MG/KG, I.V.) ON ACETYLCHOLINESTERASE ACTIVITY IN MOUSE BRAIN

TIME (MIN)	% INHIBITION
0.5	98 ± 2
5.0	95 ± 3
15.0	97 ± 0
30.0	95 ± 1
45.0	96 ± 2
60.0	96 ± 1
24 (HRS)	85 ± 3
4 DAYS	59 ± 1
7 DAYS	45 ± 2
14 DAYS	30 ± 2



EFFECT OF VARIOUS DOSES OF DFP ON ACETYLCHOLINESTERASE ACTIVITY IN MOUSE BRAIN[A]

DFP (MG/KG)	% INHIBITION
0.5	41.6 ± 3.2
1.0	67.0 ± 1.2
2.0	91.8 ± 1.5

[A] MICE WERE SACRIFICED 10 MIN AFTER DFP ADMINISTRATION

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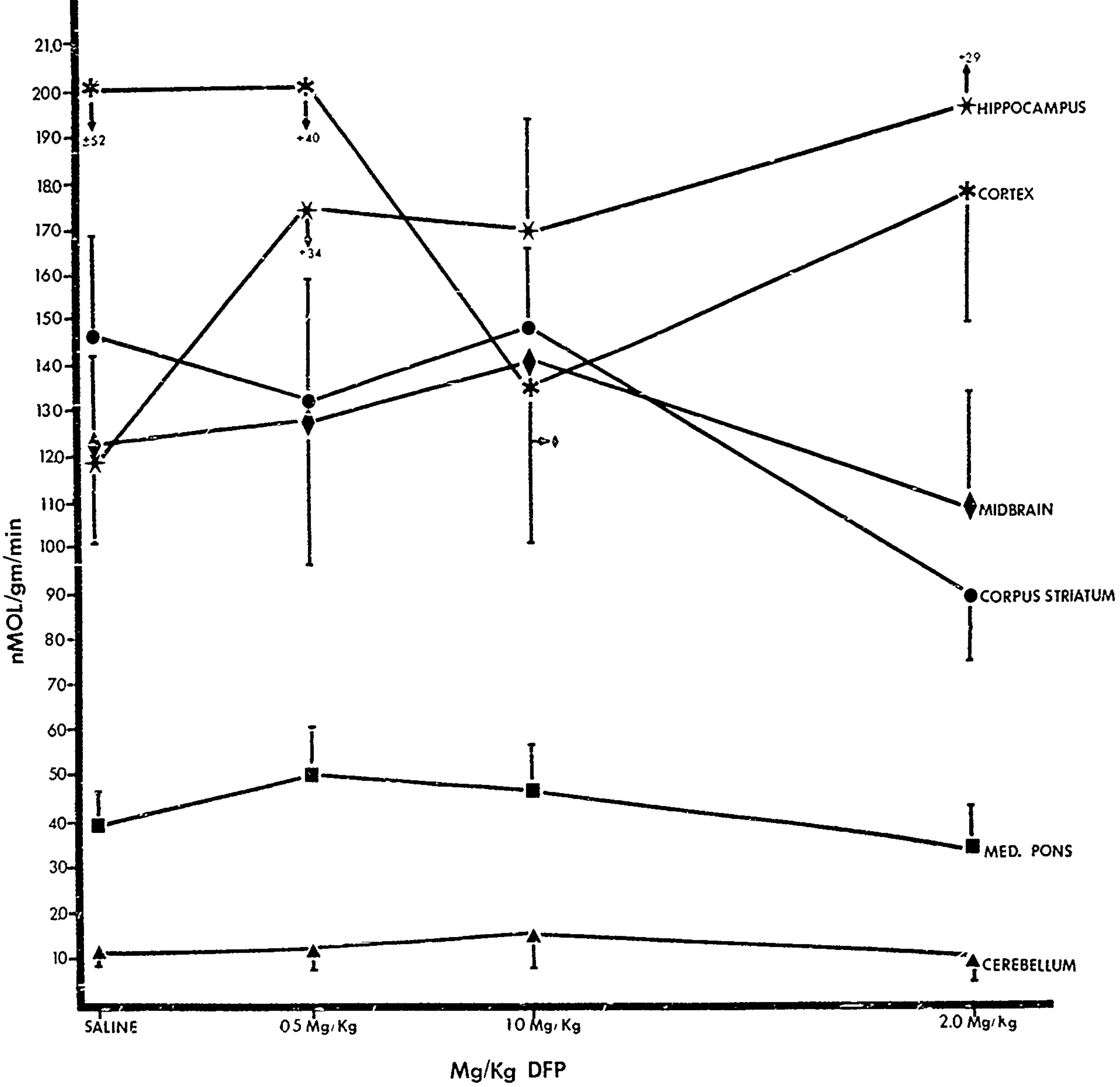
EFFECT OF DFP ON TURNOVER RATE OF ACETYLCHOLINE IN MOUSE BRAIN REGIONS



SUMMARY

EFFECT OF DFP

	PEAK	DURATION
INHIBITION OF ACHE	0.5 MIN TO: 1 HR	> 14 DAYS
SPONTANEOUS ACTIVITY	5 MIN TO 15 MIN	35 MIN
HYPOTHERMIA	1 TO 2 HRS	> 5 HRS

ACETYLCHOLINE LEVELS WERE INCREASED IN WHOLE MOUSE BRAIN AND IN MIDBRAIN AND CORTEX. CHOLINE LEVELS AND ACETYLCHOLINE TURNOVER WERE NOT ALTERED AT A DOSE OF DFP THAT INHIBITED ACETYLCHOLINESTERASE ACTIVITY BY MORE THAN 95%.

A-1365

CONCLUSION

THE LACK OF CORRELATION BETWEEN THE PHARMACOLOGICAL EFFECTS AND CHOLINERGIC CHANGES INDUCED BY DFP SUGGEST THAT NEUROCHEMICAL CHANGES OTHER THAN CHOLINERGIC ARE INVOLVED IN THE LONG TERM EFFECTS OF SINGLE DOSES OF DFP.

BIOCHEMICAL AND BEHAVIORAL RESPONSES TO INTRASTRIATAL INJECTION OF ORGANOPHOSPHATE CHOLINESTERASE INHIBITORS

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The effect of bilateral intrastriatal injection of three irreversible organophosphate cholinesterase inhibitors, DFP, soman and sarin, was studied on locomotor activity in male Sprague-Dawley rats at three time points. The degree of cholinesterase inhibition was monitored by the Ellman method (Biochem. Pharmacol. 7: 88, 1961) in the striatum, as well as in other brain areas (parietal cortex, hippocampus, hypothalamus, medulla/pons, amygdala) and trunk blood in order to ascertain the selectivity of the treatment and to rule out effects attributable to actions in these areas and/or the periphery. Locomotor activity was measured by means of capacitance-coupled activity chambers which measured both vertical and horizontal movements. The organophosphate cholinesterase inhibitors were injected into unanesthetized rats via internal cannulas inserted through previously-implanted guide cannulas such that injections were made at the coordinates of AP + 7.9, L ± 3.0, DV − 0.6 (according to Konig and Klippel) in the striata. Cholinesterase activity and locomotor activity were measured 20 min, 1 hour and 24 hrs later. Different groups of rats were used for each time point and for biochemical and behavioral testing. DFP, soman and sarin all produced a dose-dependent inhibition of striatal cholinesterase activity with a potency order of soman > sarin > DFP. DFP appeared to diffuse through the brain parenchyma more than soman and sarin, and the latter two compounds appeared to rather easily enter the peripheral circulation. However, no gross signs of peripheral cholinesterase inhibition (i.e. salivation, diarrhea) were observed. Locomotor activity was measured at doses which produced a relatively selective inhibition of striatal cholinesterase activity (to ≤ 40% vehicle control) with the cholinesterase activity of other brain areas remaining at ≥ 59% vehicle control. Locomotor activity was reduced significantly 20 min after bilateral intrastriatal administration of 81.5 nmol of DFP ($p < 0.01$). However, behavioral recovery preceded enzyme recovery because locomotor activity was not significantly different from vehicle values 1 hr and 24 hr after bilateral administration of DFP, while striatal cholinesterase activity remained 42% control at 1 hour after injection. In spite of inhibition of striatal cholinesterase activity by 60% or more, soman (14.85 nmol) and sarin (24.2 nmol) did not affect locomotor activity. Therefore, the depression of locomotor activity by DFP may not be due to cholinesterase inhibition, may be due to inhibition of one particular isoenzyme of cholinesterase or may be due to inhibition of cholinesterase in a brain region other than the striatum, as DFP does appear to produce greater cholinesterase inhibition in other brain areas than do soman and sarin. Studies of the turnover of acetylcholine and other neurotransmitters may help clarify the mechanism by which locomotor activity returns to control values. (This work was supported in part by the U.S. Army Medical Research and Development Command under contract DAMD-17-83-C-3183).

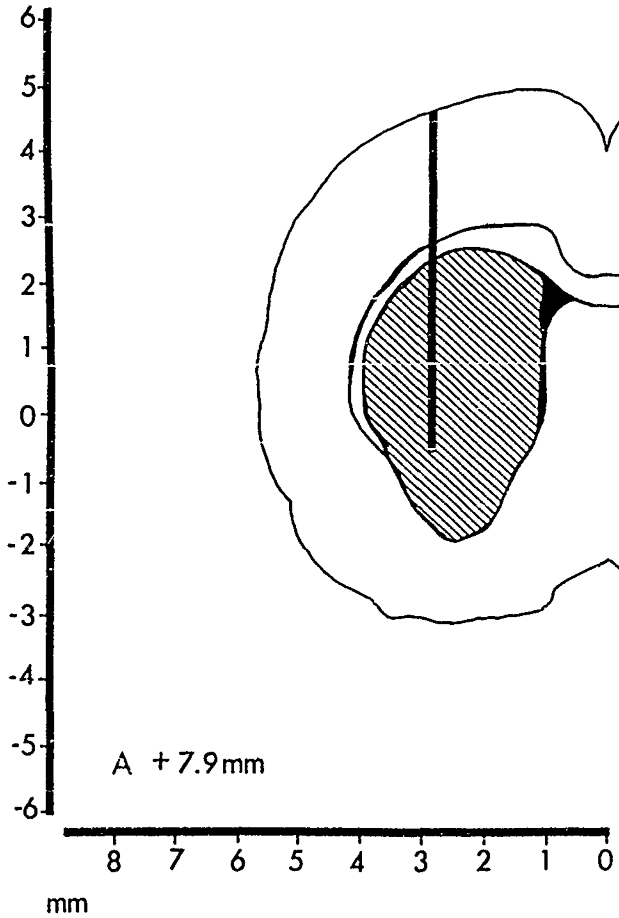

FIGURE 1. SITE OF BILATERAL INJECTION OF ORGANOPHOSPHATES IN RAT STRIATUM (AP + 7.9, L ± 3.0, V - 0.6).

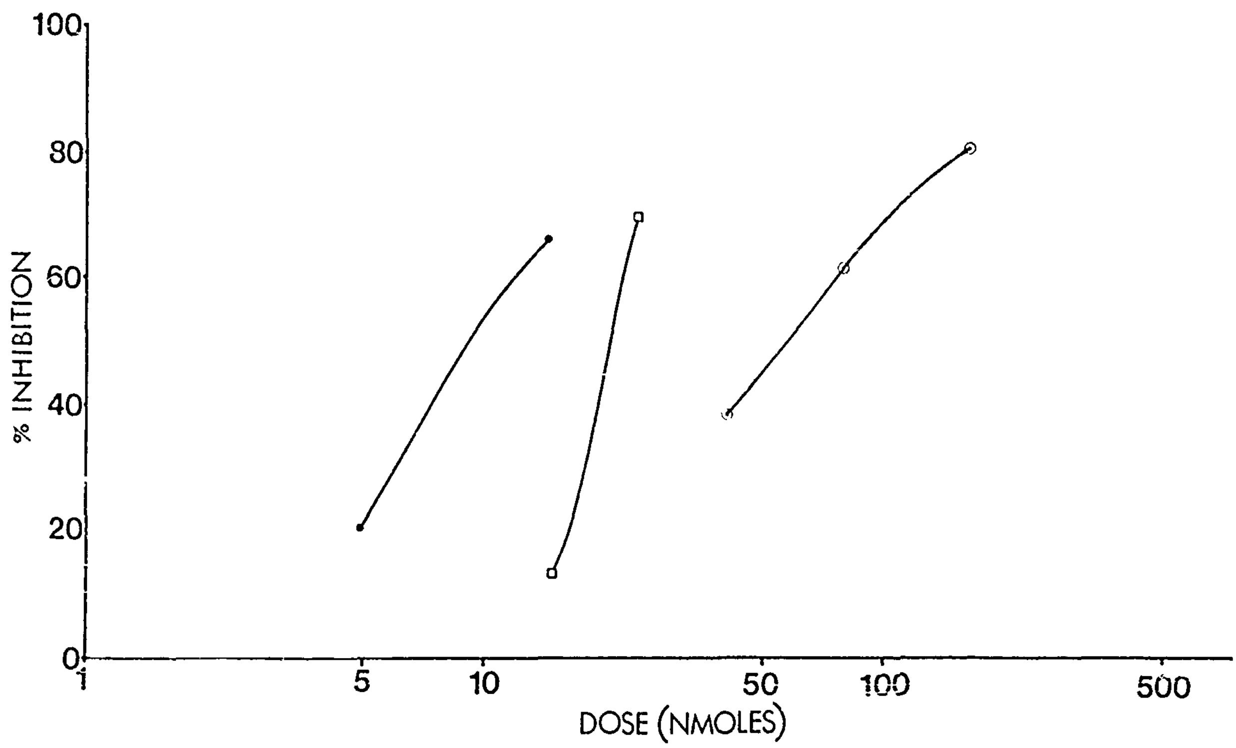

FIGURE 2. DOSE-RESPONSE CURVES FOR INHIBITION OF STRIATAL CHOLINESTERASE ACTIVITY 20 MIN AFTER INTRASTRIATAL INJECTION OF DFP (⊙), SOMAN (•) OR SARIN (□).

CHOLINESTERASE ACTIVITY AT VARIOUS TIMES AFTER BILATERAL INTRASTRIATAL INJECTION OF DFP (81.5 NMOL)

TREATMENT	(N)	BLOOD	STRIATUM	MEDULLA/ PONS	HYPOTHALAMUS	PARIETAL CORTEX	HIPPOCAMPUS	AMYGDALA
				Acetylthiocholine hydrolyzed per min				
		μmol/ml			μmol/g			
20-MIN								
VEHICLE*	(5)	1.3±0.1	36.9±3.0	12.7±0.5	7.6±0.2	5.0±0.5	8.0±0.2	12.5±1.2
DFP	(6)	1.1±0.1	14.2±2.0****	8.8±0.8	6.2±0.2****	2.9±0.3***	6.3±0.1****	9.1±1.2
(% Control)		(85%)	(38%)	(69%)	(79%)	(59%)	(79%)	(72%)
1-HR								
VEHICLE*	(5)	1.4±0.1	43.8±2.1	15.5±0.7	10.3±0.3	6.6±0.6	10.7±0.2	16.3±0.8
DFP	(6)	1.3±0.1	18.4±1.6****	10.9±0.9***	7.0±0.6****	3.9±0.5***	7.6±0.5****	12.5±0.8***
(% Control)		(93%)	(42%)	(70%)	(68%)	(59%)	(71%)	(77%)
24-HR								
VEHICLE*	(6)	1.8±0.3	37.7±2.7	14.6±0.7	9.9±0.5	5.5±0.2	8.9±0.2	18.5±0.9
DFP	(6)	0.9±0.0**	23.3±3.3***	10.7±0.5**	6.8±0.4****	3.9±0.2****	6.2±0.5****	10.8±1.0****
(% Control)		(51%)	(62%)	(74%)	(69%)	(72%)	(70%)	(58%)

* 0.5μl of emulphor: artificial CSF (1:9)
** $p < 0.05$, as compared to control.
*** $p < 0.01$, as compared to control.
**** $p < 0.001$, as compared to control.

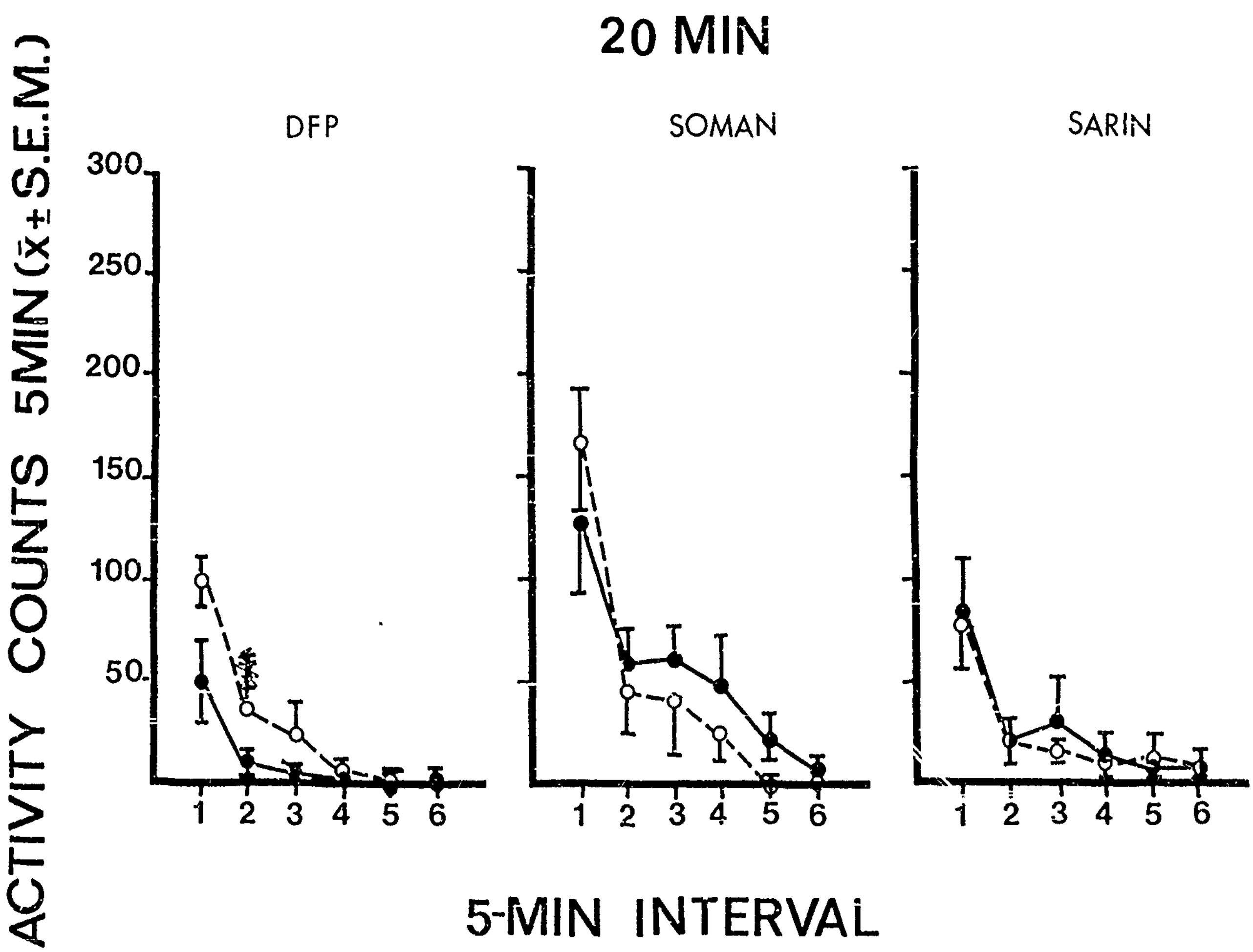

FIGURE 3. MEAN NUMBER OF ACTIVITY COUNTS PER 5 MIN INTERVAL 20 MIN AFTER INTRASTRIATAL DFP (81.5 NMOL), SOMAN (14.85 NMOL) AND SARIN (24.2 NMOL) WHERE DRUG IS (●) AND VEHICLE (○).

CHOLINESTERASE ACTIVITY AT VARIOUS TIMES AFTER
BILATERAL INTRASTRIATAL INFUSION OF 14.85 NMOLES SOMAN

TREATMENT	(N)	BLOOD	STRIATUM	MEDULLA/ PONS	HYPOTHAL- AMUS	PARIETAL CORTEX	HIPPO- CAMPUS	AMYGDALA
		Acetylthiocholine hydrolyzed per min						
		μmol/ml			μmol/g			
20-MIN								
VEHICLE*	(6)	2.1±0.3	39.1±1.1	15.3±0.3	9.0±0.4	5.5±0.1	9.1±0.4	12.2±0.5
SOMAN	(6)	0.6±0.1****	13.4±2.5****	14.0±0.3***	8.0±0.8	4.4±0.3****	8.7±0.3	10.7±1.2
(% Control)		(29%)	(34%)	(91%)	(88%)	(80%)	(96%)	(88%)
1-HR								
VEHICLE*	(6)	1.7±0.1	38.5±1.2	11.4±0.4	7.3±0.1	4.2±0.2	7.0±0.6	5.0±0.8
SOMAN	(6)	1.0±0.1****	10.7±2.5****	9.6±0.9	5.0±0.7***	2.6±0.2****	5.5±0.4	5.8±1.2
(% Control)		(59%)	(28%)	(85%)	(69%)	(62%)	(78%)	(115%)
24-HR								
VEHICLE*	(6)	1.1±0.1	36.2±1.1	11.3±0.5	10.0±0.9	4.0±0.2	6.9±0.2	10.9±0.9
SOMAN	(6)	0.5±0.1****	12.0±0.8****	12.3±0.7	6.2±0.3***	2.9±0.2***	6.1±0.3**	8.2±1.2
(% Control)		(44%)	(33%)	(109%)	(62%)	(72%)	(88%)	(75%)

* 1.5μl of sterile saline.
** $p < 0.05$, as compared to control.
*** $p < 0.01$, as compared to control.
**** $p < 0.001$, as compared to control.

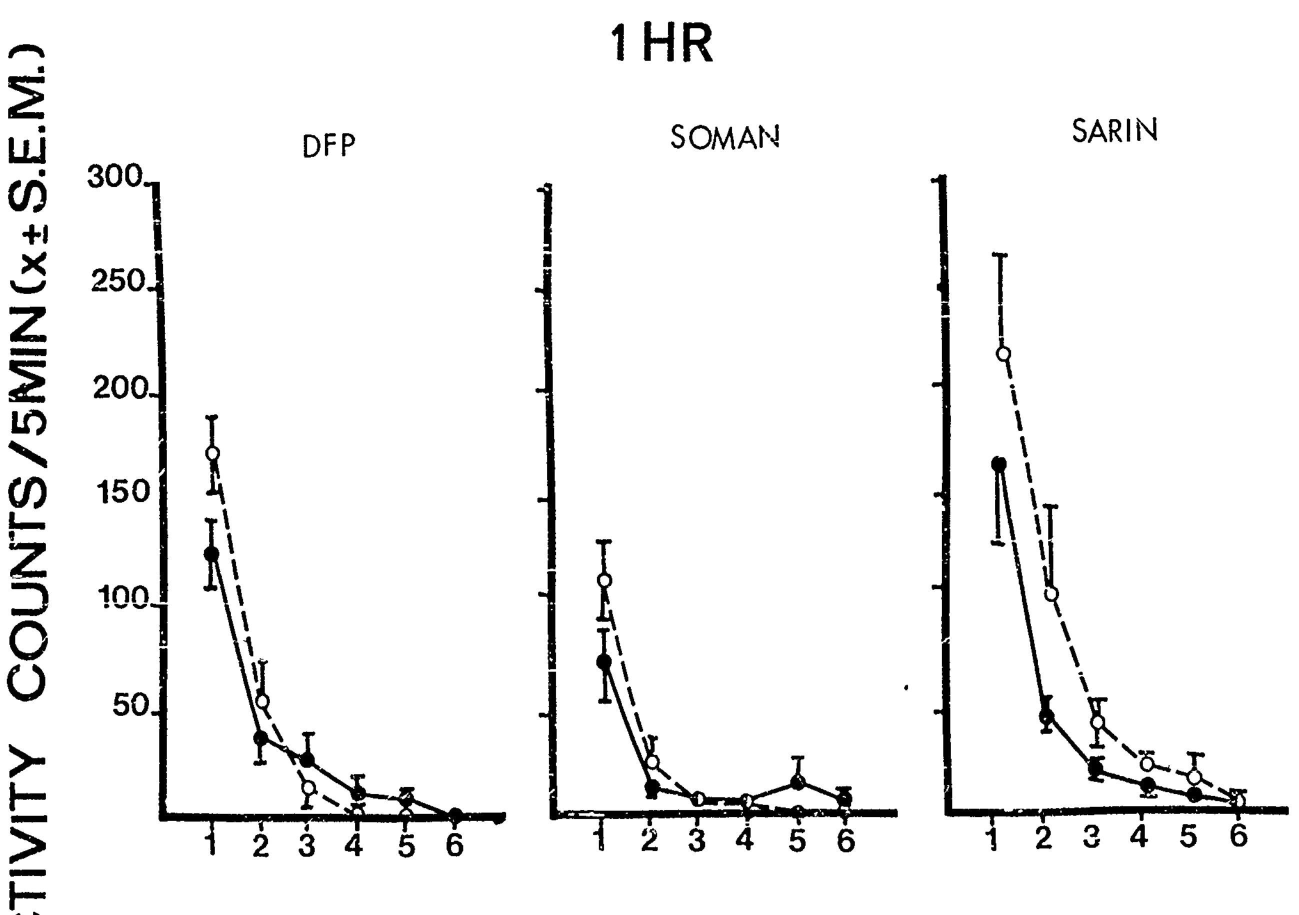

FIGURE 4. MEAN NUMBER OF ACTIVITY COUNTS PER 5 MIN INTERVAL 1 HR AFTER INTRASTRIATAL DFP (81.5 NMOL), SOMAN (14.85 NMOL) AND SARIN (24.2 NMOL) WHERE DRUG IS (●) AND VEHICLE (○).

CHOLINESTERASE ACTIVITY AT VARIOUS TIMES AFTER BILATERAL INTRASTRIATAL INFUSION OF 24.2 NMOLES SARIN

TREATMENT	(N)	BLOOD	STRIATUM	MEDULLA/ PONS	HYPOTHAL- AMUS	PARIETAL CORTEX	HIPPO- CAMPUS	AMYGDALA
		Acetythiocholine hydrolyzed per min						
		μmol/ml			μmol/g			
20-MIN								
VEHICLE*	(6)	1.1±0.1	41.8±1.9	13.9±0.6	7.9±0.2	4.3±0.2	8.2±0.3	12.0±0.5
SARIN	(6)	0.3±0.0****	12.3±2.0****	13.2±0.6	7.1±0.8	3.4±0.2***	7.2±0.3**	13.0±0.9
(% Control)		(27%)	(30%)	(95%)	(91%)	(79%)	(88%)	(108%)
1-HR								
VEHICLE*	(6)	1.1±0.1	33.9±2.3	13.1±0.5	7.6±0.8	4.2±0.5	8.2±0.8	10.6±1.2
SARIN	(6)	0.6±0.1****	20.9±2.7***	13.0±0.8	7.1±0.4	3.4±0.2	7.4±0.7	7.0±0.8**
(% Control)		(53%)	(62%)	(99%)	(94%)	(79%)	(91%)	(66%)
24-HR								
VEHICLE*	(5)	1.2±0.1	34.1±2.2	11.9±0.5	7.3±0.4	4.0±0.2	7.2±0.5	11.3±0.6
SARIN	(6)	0.6±0.1***	23.3±2.2***	10.4±0.5	6.3±0.5	3.7±0.2	7.4±0.6	11.5±1.4
(% Control)		(50%)	(68%)	(88%)	(86%)	(92%)	(102%)	(102%)

* 2 μl sterile saline.
** $p < 0.05$, as compared to control.
*** $p < 0.01$, as compared to control.
**** $p < 0.001$, as compared to control.

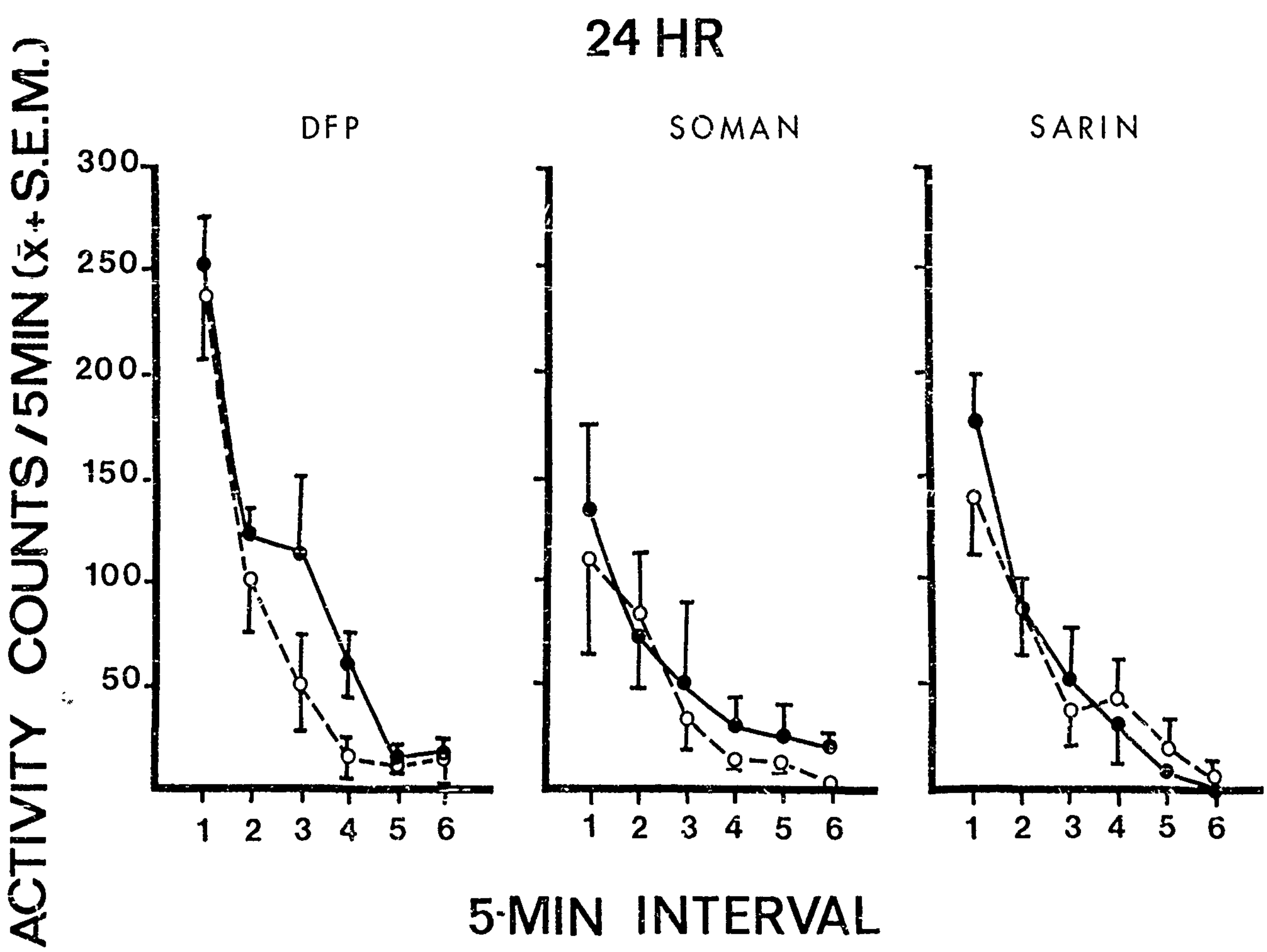

FIGURE 5. MEAN NUMBER OF ACTIVITY COUNTS PER 5 MIN INTERVAL 24 HR AFTER INTRASTRIATAL DFP (81.5 NMOL), SOMAN (14.85 NMOL) AND SARIN (24.2 NMOL) WHERE DRUG IS (●) AND VEHICLE (○).

CONCLUSIONS

DFP, BUT NOT SOMAN OR SARIN, REDUCES LOCOMOTOR ACTIVITY WHEN STRIATAL CHOLINESTERASE ACTIVITY IS INHIBITED BY APPROXIMATELY 60% IN TWENTY MINUTES, BUT NOT 1 OR 24 HOURS, AFTER BILATERAL INTRASTRIATAL ADMINISTRATION. THEREFORE, THE DEPRESSION OF LOCOMOTOR ACTIVITY:

1) IS NOT DUE TO CHOLINESTERASE INHIBITION

<u>OR</u>

2) IS DUE TO INHIBITION OF ONE PARTICULAR ISOENZYME OF CHOLINESTERASE

<u>OR</u>

3) IS DUE TO INHIBITION OF CHOLINESTERASE IN A BRAIN AREA OTHER THAN THE STRIATUM.

ATROPINE INCREASES SENSITIVITY TO STRESS IN RATS

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INTRODUCTION

Since soldiers self-administering atropine as a nerve agent antidote will also be stressed, we initiated experiments to study the potential interaction between atropine and stress. We have previously shown that acute stress increases levels of pituitary cyclic AMP and plasma prolactin in vivo in rats and that these increases are proportional to the severity of the stressor. Accordingly, rats pretreated with atropine were subjected to various stressors and levels of pituitary cyclic AMP and plasma prolactin were determined.

In the first experiment, saline injection, conditioned psychological stress, forced running, immobilization and footshock all acutely increased levels of pituitary cyclic AMP as compared to controls. Atropine-pretreated (60mg/kg) rats demonstrated higher pituitary cyclic AMP levels than saline-pretreated rats after all five stressors with statistically significant differences found after injection, immobilization and shock stressors. In the second experiment rats pretreated with 5, 10, 30 or 60 mg/kg atropine sulfate showed an increased pituitary cyclic AMP response to footshock with statistically significant differences observed between the 30 and 60 mg/kg groups vs saline-pretreated rats subjected to footshock. Plasma prolactin response to footshock was also increased in all atropine-pretreated rats with statistically significant differences observed between the 5, 30 and 60 mg/kg groups vs saline-pretreated rats subjected to footshock. In a third experiment, atropine-pretreated rats exhibited a significantly decreased latency between application of heat source and tail flick as compared to saline-pretreated rats, demonstrating that atropine lowered the pain threshhold.

Finally, in a fourth experiment, we compared the effects of atropine sulfate (a centrally acting muscarinic blocker) with the effects of atropine methyl nitrate (a peripherally acting anticholinergic drug) in order to determine whether the mechanism for the observed effects of atropine on the stress response was peripheral or central.

These data suggest that atropine potentiates neurochemical and neuroendocrine responses to stressors.

METHODS

EXPERIMENT 1. EFFECTS OF 60 mg/kg ATROPINE SULFATE ON STRESS RESPONSE

1. RATS WERE INJECTED WITH SALINE OR 60 mg/kg ATROPINE SULFATE.
2. 15 MIN. LATER RATS WERE EITHER:
   A. ALLOWED TO REMAIN IN HOME CAGE [INJECTED].
   B. SUBJECTED TO FORCED RUNNING IN A MOTORIZED ACTIVITY WHEEL [FORCED RUNNING].
   C. SUBJECTED TO FOOTSHOCK ON A VARIABLE INTERVAL SCHEDULE (30 SHOCKS/15 MIN; 5 SEC SHOCK DURATION, 1.6 mA), [FOOTSHOCK].
   D. PLACED IN A SHOCK BOX WHERE THE RAT HAD RECEIVED SHOCK ON 4 PREVIOUS DAYS BUT WITHOUT SHOCK BEING TURNED ON THIS SESSION [CONDITIONED PSYCHOLOGICAL STRESS].
   E. IMMOBILIZED IN A 5.7 CM TUBE [IMMOBILIZATION].
   F. AN ADDITIONAL CONTROL GROUP WAS SACRIFICED DIRECTLY FROM THE HOME CAGE WITHOUT ANY INJECTION [CONTROLS].
3. FOLLOWING 15 MIN OF STRESSOR, ANIMALS WERE SACRIFICED BY HIGH POWER MICROWAVE IRRADIATION. RATS WERE THEN DECAPITATED, PITUITARIES REMOVED, WEIGHED, SONICATED IN 1 ML OF BUFFER AND CENTRIFUGED. SUPERNATANTS WERE ASSAYED FOR CYCLIC AMP BY RADIOIMMUNOASSAY.

EXPERIMENT 2. ATROPINE DOSE RESPONSE AND FOOTSHOCK

1. RATS WERE INJECTED WITH EITHER SALINE OR 5, 10, 30 OR 60 mg/kg ATROPINE SULFATE.
2. 15 MIN LATER RATS WERE SUBJECTED TO FOOTSHOCK AS ABOVE.
3. FOLLOWING 15 MIN OF SHOCK RATS WERE SACRIFICED BY MICROWAVE IRRADIATION. PITUITARY EXTRACTS WERE ASSAYED FOR CYCLIC AMP AS DESCRIBED ABOVE. IN ADDITION TRUNK BLOOD WAS COLLECTED AND THE PLASMA WAS ASSAYED FOR PROLACTIN.

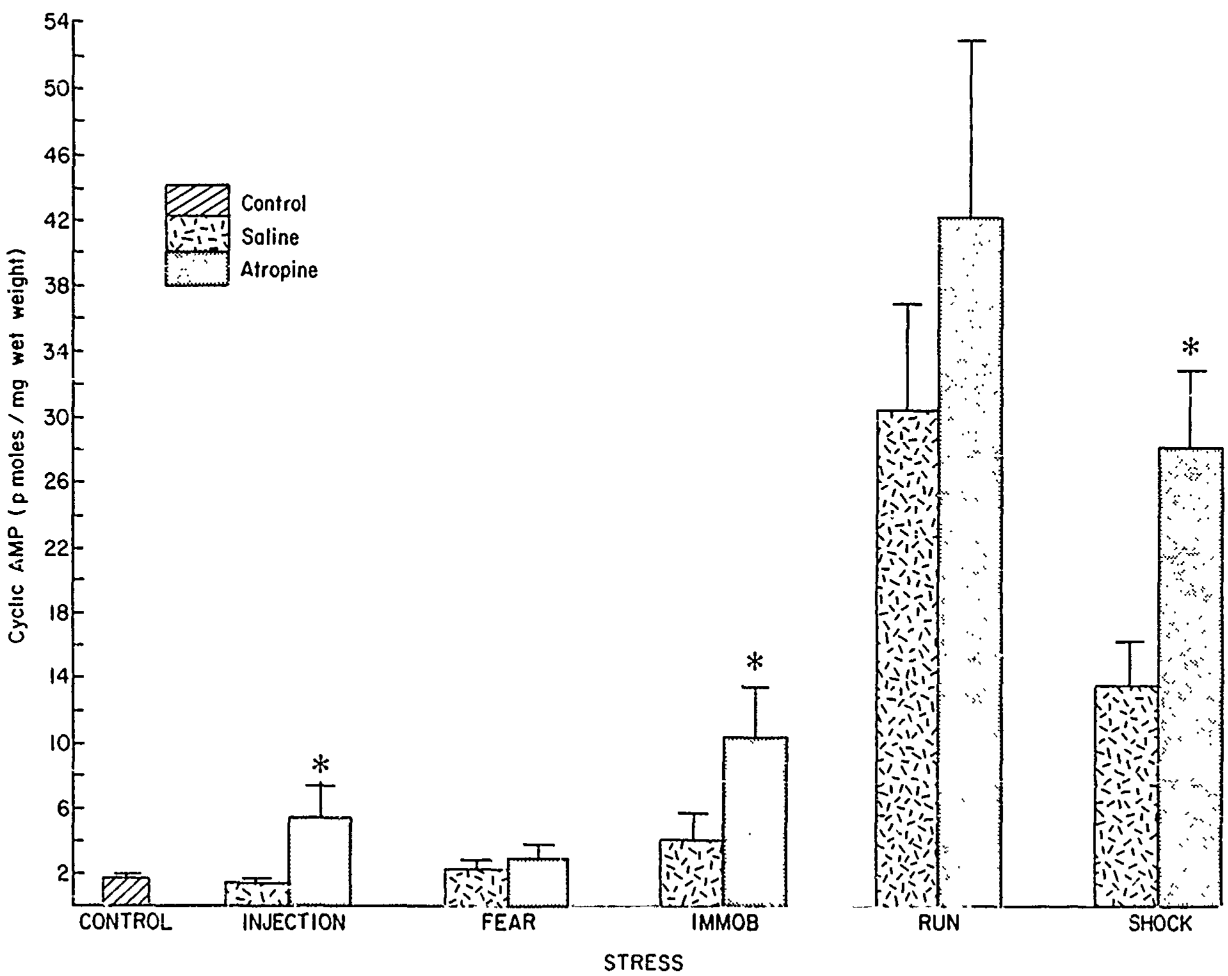
EXPERIMENT 3. EFFECT OF ATROPINE ON TAIL FLICK LATENCY

1. RATS WERE TESTED WITH TAIL FLICK APPARATUS AND BASELINE LATENCIES ESTABLISHED.
2. RATS WERE THEN INJECTED WITH EITHER SALINE OR ATROPINE SULFATE, 60 mg/kg.
3. AFTER 15 AND 30 MIN RATS WERE RETESTED FOR TAIL FLICK RESPONSE.

EXPERIMENT 4. COMPARISON OF ATROPINE SULFATE VS ATROPINE METHYL NITRATE ON STRESS RESPONSE

1. RATS WERE INJECTED WITH EITHER SALINE, 5 mg/kg ATROPINE SULFATE, OR 5 mg/kg ATROPINE METHYL NITRATE.
2. 15 MIN LATER RATS WERE SUBJECTED TO EITHER FORCED RUNNING OR IMMOBILIZATION AS ABOVE.
3. FOLLOWING 15 MIN OF STRESS RATS WERE SACRIFICED BY DECAPITATION. THE PITUITARIES WERE REMOVED, WEIGHED, AND PLACED IN 1 ML OF BUFFER AND INCUBATED IN A 90°C WATER BATH FOR 15 MIN. THE PITUITARIES WERE THEN SONICATED AND CENTRIFUGED. SUPERNATANTS WERE ASSAYED FOR CYCLIC AMP BY RADIOIMMUNOASSAY.

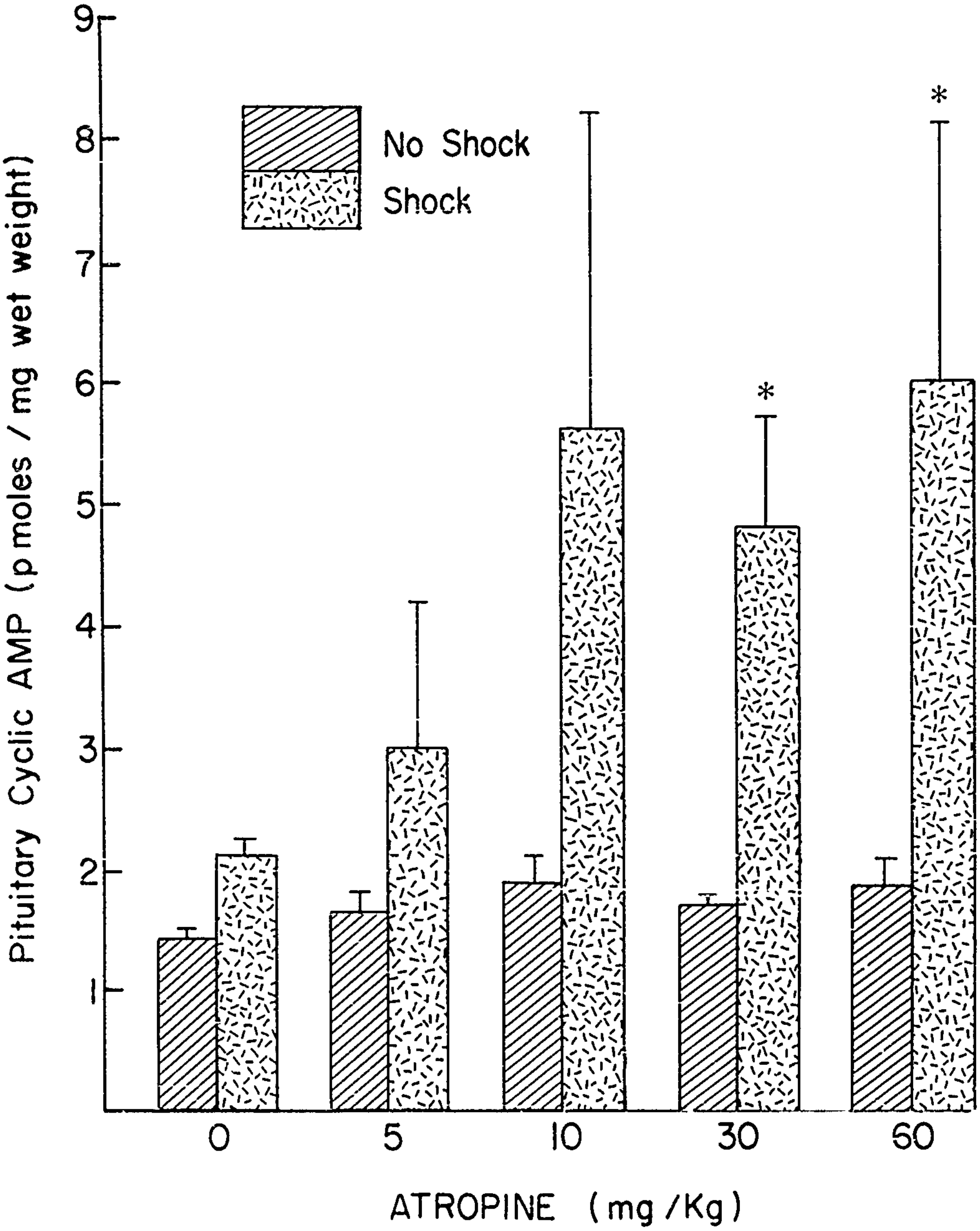
RESULTS
EXPERIMENT 1



$^*p < 0.05$, vs. SALINE–INJECTED

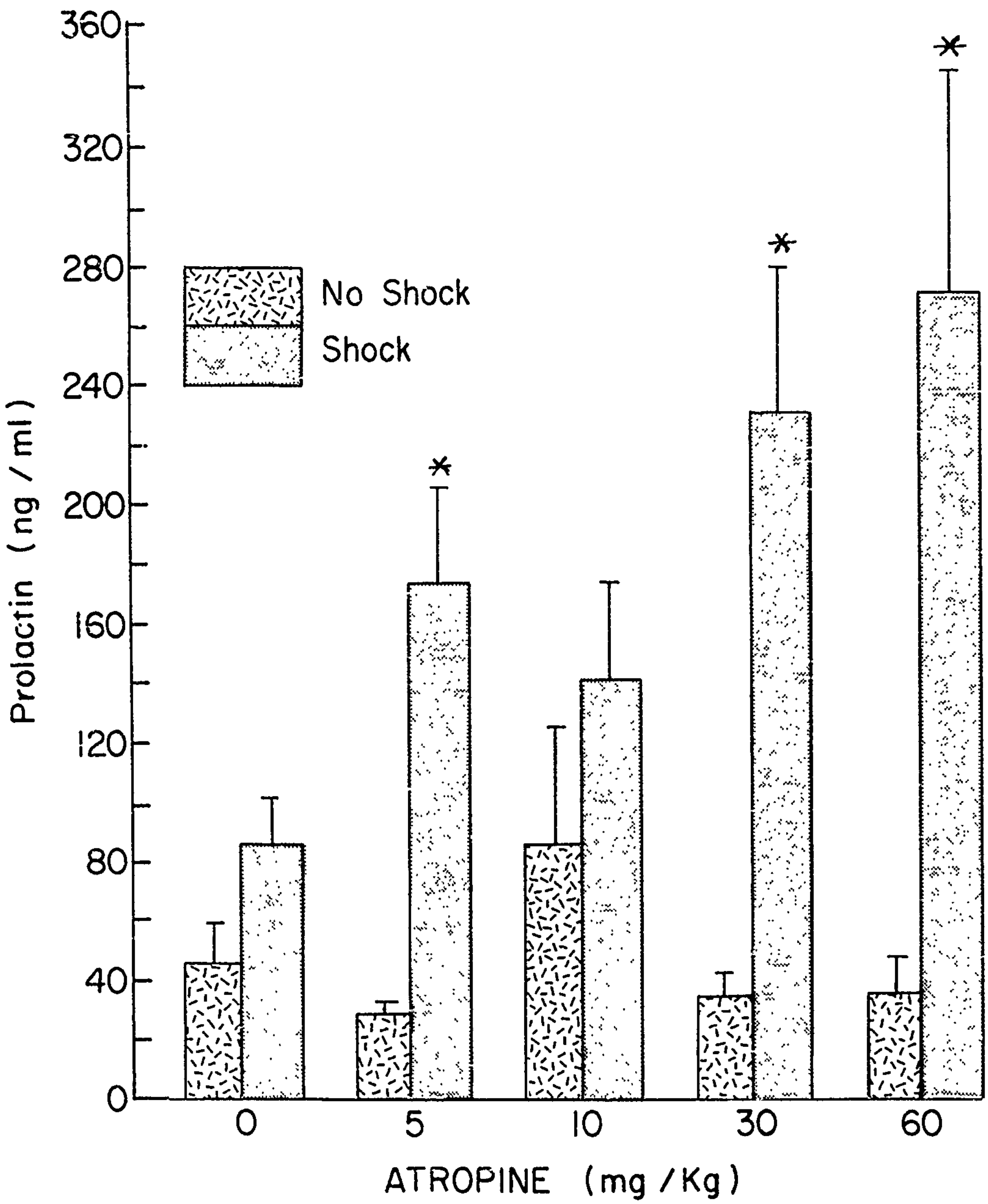
A-1375

EXPERIMENT 2



*p < 0.05, vs. NON–SHOCKED

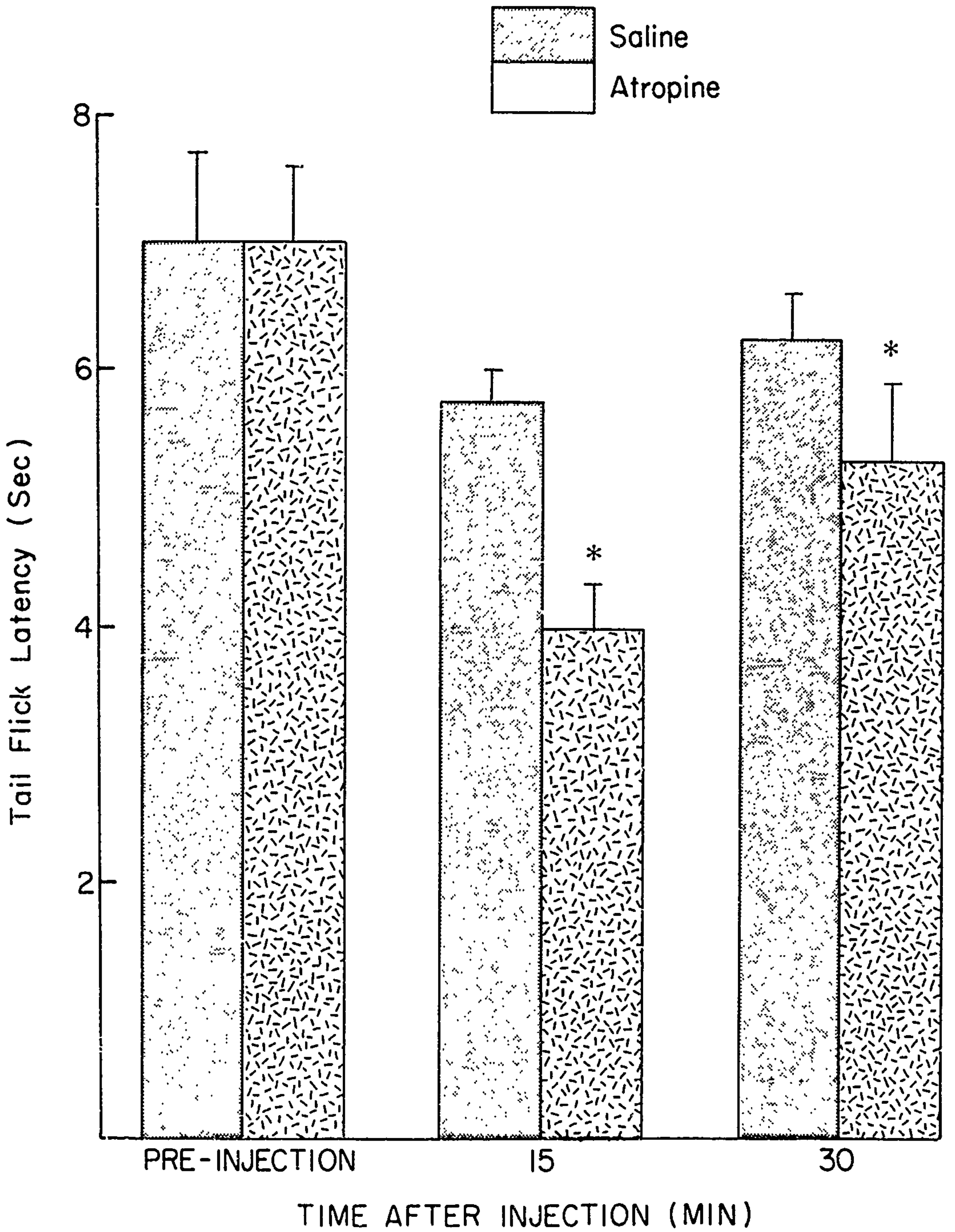
A-1376



*$p < 0.05$, vs. NON-SHOCKED

A-1377

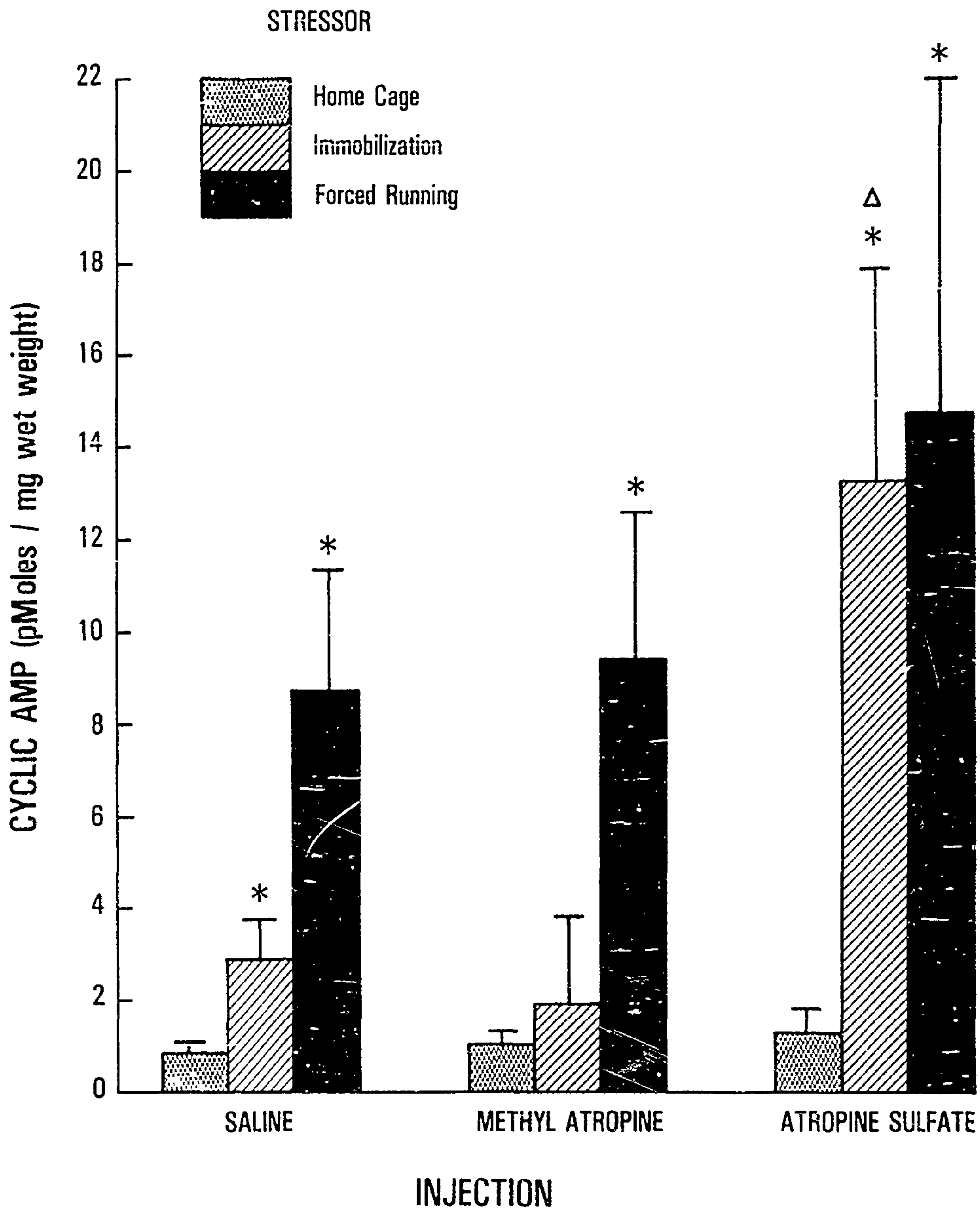
EXPERIMENT 3



***$p<0.05$, vs. SALINE–INJECTED**

A-1378

EXPERIMENT 4



***$p < 0.05$, vs. SAME DRUG / HOME CAGE**

Δ$p < 0.05$, vs. SALINE / IMMOBILIZATION

A-1379

CONCLUSIONS

1. ACUTE STRESS INCREASES PITUITARY CYCLIC AMP AND PLASMA PROLACTIN.

2. PITUITARY CYCLIC AMP RESPONSE TO 5 DIFFERENT STRESSORS WAS INCREASED IN 60 mg/kg ATROPINE SULFATE-PRETREATED ANIMALS AS COMPARED TO SALINE PRETREATED ANIMALS.

3. RATS PRETREATED WITH 5, 10, 30 OR 60 mg/kg ATROPINE SULFATE SHOWED AN INCREASED PITUITARY CYCLIC AMP RESPONSE AND PLASMA PROLACTIN RESPONSE TO FOOTSHOCK COMPARED TO SALINE PRETREATED RATS SUBJECTED TO FOOTSHOCK.

4. ATROPINE SULFATE-PRETREATED RATS EXHIBITED A DECREASED LATENCY BETWEEN APPLICATION OF HEAT SOURCE AND TAIL FLICK AS COMPARED TO SALINE-PRETREATED RATS.

5. RATS PRETREATED WITH 5 mg/kg ATROPINE SULFATE AS OPPOSED TO RATS PRETREATED WITH 5 mg/kg ATROPINE METHYL NITRATE SHOWED AN INCREASED STRESS RESPONSE TO 15 MIN OF IMMOBILIZATION, SUGGESTING THAT ATROPINE'S EFFECT ON STRESS RESPONSE OCCURS CENTRALLY RATHER THAN PERIPHERALLY.

6. THESE DATA SUGGEST THAT ATROPINE MAY ACT CENTRALLY TO POTENTIATE NEUROCHEMICAL AND NEUROENDOCRINE RESPONSE TO STRESSORS.

ATROPINE AND 2-PAM ELICIT STRESS-INDUCED CONVULSIONS IN MICE

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INTRODUCTION

Atropine sulfate and 2-pyridine aldoxime methylchloride (2-PAM) are currently the treatments of choice for acute organophosphate intoxication. In addition to their pharmacological effectiveness, these drugs have the advantage of being relatively non-toxic even after repeated administration of high doses in both man and animals. However, most toxicological studies to date have been performed under stable environmental conditions which may not always be attainable. For example, the potential use of these antidotes during chemical warfare operations would occur under stressful environmental situations.

Stress has been shown to modify the pharmacological and toxicological actions of some drugs. Thus, it is conceivable that the toxicity of atropine and 2-PAM may be altered by stress, resulting in adverse behavioral changes not previously observed. Therefore, the present study was designed to: 1) examine a possible drug x stress interaction with atropine and 2-PAM during an intense, uncontrollable stressful situation and 2) investigate the mechanism(s) responsible for such changes, if present.

METHODS

Male ICR Swiss mice weighing 20-25 g were used throughout this study.

Swim Stress Test. Mice were injected with either vehicle or drug and then placed individually in 2 liter beakers filled 15 cm high with water (16°C). Under these conditions each mouse could swim freely about the beaker in a coordinated fashion but could not escape. The animals were left in the beakers until showing signs of fatigue or any adverse behavioral effects.

Rectal Body Temperature. Digital microprobe theromometers containing ball-tipped rectal probes were used to monitor internal body temperature. Each mouse was used as its own control throughout a particular experiment.

Monitoring Plasma Glucose Levels. Blood samples were taken by clipping the end of the tail and collecting blood in heparinized capillary tubes. Following centrifugation, 20 μl of plasma was used to determine the glucose concentrations by a commercially available assay which utilizes the glucose oxidase method.

Statistics. Analysis of frequency data was performed using the G-test of independence with Williams' correction. Changes in body temperature were analyzed using analysis of variance for repeated measures, followed by Fisher's least significance difference test for group comparisons. Plasma glucose levels were analyzed using analysis of variance, followed by Newman-Keuls multiple range test to compare different groups. Data are expressed as the mean $\pm$ SEM.

TABLE 1
BEHAVIORAL EFFECTS OF ATROPINE AND 2-PAM DURING COLD WATER SWIMMING

N	ATROPINE (mg/kg)	2-PAM (mg/kg)	% CONVULSIONS	LATENCY TO CONVULSIONS (minutes)	% DIED
6	150	-	83	8.6 ± 1.2	50
6	80.6	-	83	14.7 ± 1.2	33
12	11.2	-	0	-	0
6	5.6	-	0	-	0
6	0.1	-	0	-	0
6	-	100	83	7.3 ± 1	83
6	-	50	33	11.6	33
19	-	25	0	-	0
6	-	12.5	0	-	0
6	-	5	0	-	0
6	150	100	100	1.9 ± 0.2	100
6	150	50	100	3.7 ± 0.8	100
6	150	25	100	4.5 ± 0.9	100
8	11.2	25	75	15.2 ± 2.8	50
8	11.2	12.5	13	19.9	0
8	11.2	5	0	-	0
8	5.6	100	88	3.3 ± 0.8	88
8	0.1	12.5	13	20.9	13
8	0.1	5	0	-	0

Mice were injected i.m. with atropine and/or 2-PAM immediately prior to performing the swim stress test. N = number of observations. Control animals (N = 44) receiving only vehicle never convulsed or died.

TABLE 2
BEHAVIORAL EFFECTS OF N-METHYL ATROPINE AND 2-PAM DURING COLD WATER SWIMMING

N	N-METHYL ATROPINE (mg/kg)	2-PAM (mg/kg)	% CONVULSIONS	LATENCY TO CONVULSIONS (minutes)	% DIED
5	150	-	100	4.1 ± 0.4	100
5	80.6	-	100	6.3 ± 0.6	80
5	11.2	-	0	-	0
6	11.2	25	66	10.1 ± 1.0	66

Mice were injected i.m. with N-methyl atropine, alone or in combination, with 2-PAM prior to performing the swim stress test. N = number of observations. Control animals (N = 6) receiving only vehicle never convulsed or died.

TABLE 3
EFFECTS OF DIAZEPAM ON ATROPINE AND 2-PAM STRESS-INDUCED CONVULSIONS

N	DIAZEPAM (mg/kg)	ATROPINE + 2-PAM (mg/kg)		% CONVULSIONS	LATENCY TO CONVULSIONS (minutes)	% DIED
16	-	11.2	25	100	10.5 ± 1.0	63
10	1.25	11.2	25	70*	11.0 ± 0.7	20*

Mice were injected i.m. with vehicle or diazepam, followed immediately by an i.m. injection of atropine and 2-PAM. The mice were placed in the beakers to perform the swim stress test. N = number of observations. * $P < 0.05$ with respect to atropine and 2-PAM alone.

TABLE 4
EFFECTS OF PILOCARPINE ON ATROPINE AND 2-PAM STRESS-INDUCED CONVULSIONS

N	PILOCARPINE (mg/kg)	ATROPINE + 2-PAM (mg/kg)		% CONVULSIONS	LATENCY TO CONVULSIONS (minutes)	% DIED
10	-	11.2	25	90	11.1 ± 1.0	70
10	5	11.2	25	80	11.9 ± 0.6	10*
10	-	80.6		70	16.0 ± 1.0	20
10	5	80.6		90	15.2 ± 2.1	30

Mice were pretreated with an i.m. injection of vehicle or pilocarpine 10 minutes prior to the i.m. administration of atropine, alone or in combination, with 2-PAM. The mice were placed in the beakers to perform the swim stress test. N = number of observations. * $P < 0.05$ with respect to atropine and 2-PAM alone.

TABLE 5

EFFECTS OF PHENOXYBENZAMINE (PBZ) ON ATROPINE AND 2-PAM STRESS-INDUCED CONVULSIONS

N	PBZ (mg/kg)	ATROPINE + 2-PAM (mg/kg)		% CONVULSIONS	LATENCY TO CONVULSIONS (minutes)	% DIED
16	–	11.2	25	38	14.2 ± 1.0	53
6	6	11.2	25	0	–	0*
10	3	11.2	25	20*	9.8	10*
10	1	11.2	25	50*	12.4 ± 2.0	40
10	–	80.6	–	80	12.2 ± 0.9	60
10	3	80.6	–	80	14.2 ± 1.2	50
10	–	–	100	90	7.5 ± 0.8	80
10	3	–	100	90	8.0 ± 0.7	90

Mice were pretreated with an i.p. injection of vehicle or PBZ one hour prior to the i.m. administration of atropine and 2-PAM. The mice were placed in the beakers to perform the swim stress test. N = number of observations. * $P < 0.05$ with respect to atropine and 2-PAM alone.

TABLE 6

EFFECTS OF PROPRANOLOL ON ATROPINE AND 2-PAM STRESS-INDUCED CONVULSIONS

N	PROPRANOLOL (mg/kg)	ATROPINE + 2-PAM (mg/kg)		% CONVULSIONS	LATENCY TO CONVULSIONS (minutes)	% DIED
11	–	11.2	25	82	12.9 ± 1.2	55
8	20	11.2	25	75	11.8 ± 1.0	50

Mice were pretreated with an i.p. injection of vehicle or propranolol 15 minutes prior to the i.m. administration of atropine and 2-PAM. The mice were placed in the beakers to perform the swim stress test. N = number of observations.

TABLE 7
EFFECTS OF ATROPINE (Atr), 2-PAM AND SWIM STRESS ON RECTAL BODY TEMPERATURE (°C)

TREATMENT (mg/kg)	POST-INJECTION TIME (minutes)					
	BEFORE	0	5	10	15	30
CONTROL	38.3 ± .1	38.7 ± .09	38.7 ± .09	38.8 ± .09	38.8 ± .1	38.6 ± .1
Atr (150)	38.3 ± .1	38.9 ± .07	38.1 ± .1	37.4 ± .1*	37.2 ± .3*	36.4 ± .1*
Atr (11.2)	38.7 ± .1	38.9 ± .07	38.9 ± .1	38.7 ± .1	38.7 ± .1	38.3 ± .06
2-PAM (100)	38.2 ± .1	38.4 ± .1	37.5 ± .1*	36.8 ± .1*	37.0 ± .2*	37.7 ± .2
2-PAM (25)	38.3 ± .1	38.4 ± .08	38.7 ± .1	39.0 ± .1	38.8 ± .1	38.6 ± .1
Atr (11.2) + 2-PAM (25)	38.4 ± .2	38.5 ± .1	38.0 ± .1	38.0 ± .2	38.1 ± .2	38.3 ± .09

	BEFORE STRESS	AFTER STRESS	% CHANGE
CONTROL	38.7 ± 0.1	21.0 ± 0.4**	46
Atr (11.2) + 2-PAM (25)	38.1 ± 0.1	23.1 ± 0.4**	39

Rectal body temperatures of mice were taken before and after the i.m. injection of vehicle, atropine and/or 2-PAM. In addition, rectal body temperature of control and drug-treated animals were also taken before and after performing the swim stress test. Doses are shown in parentheses. Six observations were made per treatment group. * $P < 0.05$ with respect to the appropriate treatment before injection. ** $P < 0.05$ with respect to the appropriate treatment before stress.

TABLE 8
EFFECTS OF ATROPINE, 2-PAM AND SWIM STRESS ON PLASMA GLUCOSE LEVELS

TREATMENT	N	PLASMA GLUCOSE LEVELS (mg/100 ml)
UNSTRESSED	7	150.6 ± 10.1
SWIM STRESS	13	169.4 ± 4.3
ATROPINE + 2-PAM + SWIM STRESS	6	122.6 ± 12.0*

Plasma glucose levels of mice were measured immediately following one of the above treatments. Doses of atropine and 2-PAM were 11.2 and 25 mg/kg, i.m., respectively. N = number of observations. * $P < 0.05$ with respect to unstressed and swim stressed treatment groups.

SUMMARY

1. Atropine and 2-PAM, in dose combinations which do not produce any adverse effects on the behavior of mice in a stable environment, elicited violent clonic-tonic convulsions and death within 15-20 minutes when the animals were stressed by cold water swimming. Thus, an adverse drug x stress interaction between atropine, 2-PAM and acute physical stress is evident.

2. Both gabaergic and alpha-adrenergic mechanisms appear to be involved in the precipitation of convulsions since diazepam and phenoxybenzamine significantly reduced or abolished the occurrence of convulsions. Although pilocarpine did not reduce the incidence of convulsions, it did produce a significant decrease in the number of deaths following the convulsions. Thus, cholinergic mechanisms also appear to be involved in this drug x stress interaction.

3. Peripheral mechanisms may be important since N-methyl atropine produced effects similar to atropine when combined with 2-PAM.

4. Changes in internal body temperature and plasma glucose levels do not appear to explain the precipitation of convulsions.